## EUROPEAN PATENT APPLICATION

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(54) **Lottery terminal.**

(57) A lottery terminal (1) for playing lottery games and for money and data transmissions is described.

Also described are methods for playing lottery or the like games using the terminal (1).
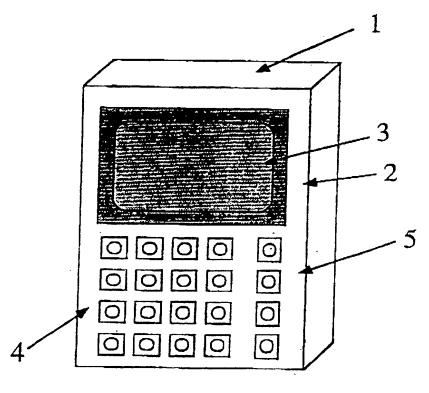
Fig. 1

EP 0 450 520 A2

The present invention relates to a lottery terminal for playing lottery games and for other purposes involving data and money transmission, and to methods of playing lottery games which employ this terminal.

Lottery games, herein defined, should be taken to include all types of games in which numbers or values are selected by the player, to match sets of numbers or values which are either predetermined or generated during the game, and should be understood to include, e.g., games such as bingo, football pool, horse racing, lotteries, such as national lotteries, and the like.

The art is constantly trying to provide means for playing lottery games which are free from the need and the limitations deriving from the existing system which requires the player to buy tickets, or to physically obtain lotteries, such as in the bingo game, so that lottery games may be played from home, at different times, and without all the existing limitations. For instance, U.K. patent applications GB 2,148,135 and 2,197,971, and European Patent Application EP 310368 describe equipment and methods using remote terminal techniques. These devices and methods, however, have the considerable drawback of requiring direct connection with computer centers or the like, require expensive and complicated equipment and networks, and do not solve all of the aforesaid problems.

It is an object of the present invention to provide an apparatus and method by means of which a player of lottery games will be able to play the game from any location, at any time, and without any substantial limitation.

It is another object of the invention to provide an apparatus by means of which transmission of data relating to various transactions, such as bank or stock orders, purchases or the like, can be easily effected.

It is a further object of the invention to provide an apparatus which is simple to operate and inexpensive, while affording a high degree of security against unauthorised uses.

The apparatus according to the invention is, in fact, a terminal, which terminal comprises a housing provided with:

- display means;
- memory means, comprising data storing, recording and retrieving means;
- data input means,
- power source; and
- data transmitting and/or receiving means to transmit data via a telephone line.

The term "wireless", whenever used herein, is meant to indicate that wire connection between the terminal and a receiving station is not necessary, but connections by means of wires to a telephone line is, of course, possible. Thus, the data transmitting and/or receiving means, such as modem means, which are necessary to transmit data through a telephone line, may certainly include direct wire connection to a telephone set or line. However, in many instances, it will be convenient to provide an acoustic coupler in the terminal, by means of which connection can be established and data transmitted through a telephone line, without any direct wire connections.

According to a preferred embodiment of the invention the memory means comprise a removable memory. As will be apparent as the specification proceeds, in many instances it will be convenient and - in some cases, it may be necessary - to provide data which are to be purchased by the player at a selling station, which may include sets of values or of data concerning a specific game or prepaid time or games data. One convenient way of providing such data is to provide removable memory means, such as a chip-card, within the memory means, which may contain prerecorded data and may also be used to store data generated during the game. Alternatively, the memory means may be fixed within the terminal, and data may be fed to it at a purchasing station directly through a connection to terminal means of the said station.

Other means, such as display means which may be, e.g., a liquid crystal display, and the data input means which may be a keyboard, are well within the scope of the skilled technician, and therefore are not described in detail. As will be understood by a skilled person, the device needs be powered, and a power source may be provided, such as an electric battery, but connection to an electric line may also be provided.

As will be understood, in many cases when playing lottery games the player is required to decide upon a very large number of data. For this purpose, a random number generator may be provided in the device of the invention, by means of which, if desired, the player may generate part - or all - of his data.

The invention further embraces a method of playing a lottery or the like game, which method comprises the steps of:

(a) providing a terminal according to the invention;

(b) providing credit codes to enable the player to play;

(c) selecting and feeding to the terminal data to be provided by the player of the game;

(d) obtaining telephone connection to a receiving center;

(e) transmitting the data provided by the player and any required identification and/or credit data to the said receiving center; and

(f) if required, receiving from the said receiving center validation data and storing the said vali-

dation data in the memory means of the terminal.

All the above and other advantages and characteristics of the invention will be better understood through the following illustrative and non-limitative description of preferred embodiments.

In Figs. 1 and 2 a device according to a preferred embodiment of the invention is schematically shown. This device, generally indicated by numeral 1, comprises a housing 2, a display 3, a keyboard 4, which may be used as an input device for numbers or alpha-numeric data, and an additional control board 5, which is optional, and which may be used, e.g., for transmission and/or other control procedures, such as for reading or storing data in the memory. The controls 5 may also be integral with the keyboard 4, and alternation switches may be provided to permit to use one key for more than one function.

In Fig. 2 the back of the device of Fig. 1 is shown, and an acoustic coupler 6 is seen, which has the shape and size adopted to be used for transmitting data through a telephone handset, in a way well known in the art. The said acoustic coupler can be replaced, whenever convenient, by a wire connection, as hereinbefore explained. Appropriate modem means can be provided, as known to persons skilled in the art. Of course, if a bi-directional communication is to be effected, to enable the device to receive and/or record data from the receiving center, appropriate receiving means and communication protocols must be provided, which are well within the scope of the skilled person and which therefore are not described herein for the sake of brevity. Power means 7 are indicated, which may comprise a battery or an input for line current, or both.

In Fig. 2 numeral 8 schematically indicates an opening for detachable memory means, e.g., a chip-card. This chip-card, indicated in the figure by numeral 9, may be introduced through slot 8, and appropriate security means may be provided to avoid the unauthorized removal or tampering with the card.

Furthermore, for various applications it may be useful to provide data input devices, such as a barcode or optical reader, e.g., to read price lists into the memory of the terminal, for telemarketing or other use. Additionally, it may be desired to print out displayed or memorized data for future reference, record keeping, etc., and a suitable printer can be added, which may be external or built-in. The addition of such devices is, of course, within the skill of the routineer, and therefore such devices are not described here in detail, for the sake of brevity.

The device described in Figs. 1 and 2, with appropriate modifications, may be used for a variety of games. A number of games will be listed below.

## Bingo game

The bingo game can be interactively played in front of a television set. The numbers are broadcast from the studio and are seen on the television screen. The device may operate in two modes: (1) a set of numbers such as on a bingo board, may appear on the display 3 and can be marked by means of the keyboard, and the player may follow the numbers broadcasted to see whether one or more of the broadcasted numbers are included in the board appearing on the display 3. (2) alternatively the player may listen to the broadcasted numbers, feed them to the device through keyboard 4, and may be alerted by the device if one of these numbers is included in the boards he has. Different boards may be retrieved from the chip-card, which card may include a number of games as purchased previously by the player. Thus, a chip-card containing a large number of different boards may be purchased and used in subsequent games. The chip-card may also contain a security number which identifies it, to avoid tampering with the data therein. When a player has "filled" his board with the broadcasted number, viz., has obtained bingo, he may dial to a receiving center which would normally be located at the studio, and inform the studio of his winning. This may be done by voice, but alternatively, the invention conveniently permits to communicate automatically with the receiving center. In this case, the player will dial the number of the receiving center, and will transmit by appropriate transmitting switches through modem 6 the data presently existing in the device. This data will include his board and the security number of the board and/or of the chip-card, and may include additional data, such as the identity number of the player or the like identification data. The receiving center may receive the board, review the data, validate the winning and, if desired, may transmit back to the transmitting device a validation number to be registered in the memory, whether in the device or in the chip-card, which will serve as proof of the winning at a later date, and may announce the winning by immediate broadcasting and, if desired, the name of the winner.

## Loto game

The loto game will slightly differ from the bingo game. In the loto game the chip-card will contain prepaid games or values or numbers, which may represent the games and/or the credit of the player, and the player will select, either manually or by

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using a random number generator, which may be integral with the device, a number or a plurality of numbers and transmit them through a telephone line as above to the receiving center, at which time the prepaid game will be detracted from the pre-paid amount found on the chip-card. Alternatively, if the prepaid games or values or numbers are not sufficient, the player may transmit credit data, such as the number of a credit card, and may play even if the prepaid games or values or numbers on the chip-card are finished.

Validation of the transmission to a receiving center may be done together with the registration of the number on the chip-card, and the data transmitted to the receiving center will be kept on record in the memory of the device, e.g., in the chip-card, for later reference. When the right num-bers are broadcasted or otherwise published, the player will feed the right numbers to the device, and the device will automatically search through all games he has played, to determine whether any winnings have occurred. The numbers may be fed by the player one by one, through the keyboard, or sets of numbers can be generated by a random number generator, provided for the convenience of the player in the device. Unlike in existing games, if a set of numbers is to be used for the game, different numbers of the same set may be decided upon and transmitted to the receiving center at different times. Thus, for instance, a partial broad-casting of winning numbers may be done at dif-ferent times, and the winnings may be different depending on the time and on the stage at which the bet is placed through the receiving center.

## Football pool (Toto)

The same device, with small modifications, may be employed for playing football pool or dif-ferent gambling games, such as horse racing, etc.. In this case a matrix of games and odds (1, 2 and X) must be provided, to enable the player to select his bets. This may be provided on the display of the device already in matrix form, or data as to the games may be supplied separately and fed linearly to the device. Here, again, random number gener-ators may be employed to decide which values to use for a given game or sets of games. The games is then continued by transmitting and/or receiving data to and from a receiving center, as explained above with respect to the Loto game, and with the required modifications, as appropriate.

All the above has been provided for the pur-pose of illustration, and is not intended to constitute a limitation of the invention. It will be apparent to a skilled person that a large variety of games may be played by means of the invention, and small modi-fications in the device, input and output thereof and method of using it will not exceed the invention.
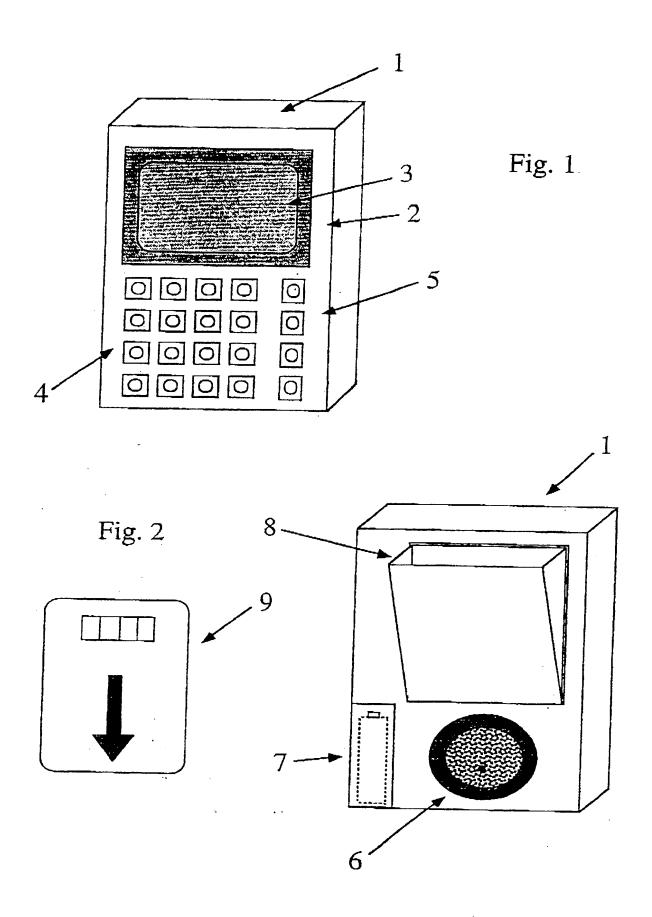
Likewise, additional uses can be devised, as will be apparent to a skilled person, such as telemarketing, bank or stock transactions and the like operations, with the changes in operation meth-ods that will easily be efected by the skilled per-son.

## Claims

1. A lottery terminal, comprising a housing pro-vided with:
   - display means;
   - memory means comprising data storing, recording and retrieving means;
   - data input means;
   - power source; and
   - data transmitting and/or receiving means to transmit data via a telephone line.

2. A terminal according to claim 1, wherein the memory means comprise a removable mem-ory.

3. A terminal according to claim 2, wherein the removable memory is a chip-card.

4. A terminal according to claim 2 or 3, wherein the removable memory comprises prepurchas-ed credit in the form of data, values or games.

5. A terminal according to any one of claims 1 to 4, wherein the data transmitting and or receiv-ing means comprise an acoustic coupler.

6. A terminal according to any one of claims 1 to 5, wherein the display means comprise a liquid crystal display.

7. A terminal according to any one of claims 1 to 6, further comprising a random number gener-ator.

8. A terminal according to any one of claims 1 to 7, further comprising a data imput device, such as a bar-code or optical reader or the like.

9. A terminal according to any one of claims 1 to 8, further comprising print-out means to print out displayed or memorized data.

10. A method of playing a lottery or the like game, comprising the steps of:
    a) providing a terminal according to any one of claims 1 to 9,
    b) providing credit codes to enable the play-er to play;
    c) selecting and feeding to the terminal data

to be provided by the player of the game;

d) obtaining telephone connection to a receiving center;

e) transmitting the data provided by the player and any required identification and/or credit data to the said receiving center; and

f) if required, receiving from the said receiving center validation data and storing the said validation data in the memory means of the terminal.

**11.** A method according to claim 10, wherein the game is selected from among the game of bingo, football pool, horse-racing and national lotteries.

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Fig. 1

Fig. 2

# EUROPEAN PATENT APPLICATION

## Lottery terminal.

A lottery terminal (1) for playing lottery games and for money and data transmissions is described. Also described are methods for playing lottery or the like games using the terminal (1).
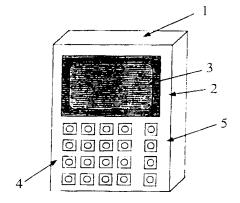
Fig. 1

# PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention
shall be considered, for the purposes of subsequent
proceedings, as the European search report

Application Number

EP 91 10 5017

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| Y,D | GB-A-2 148 135 (DICKINSON et al.) <br> * Page 2, lines 56-59,75-92; figure 1 * <br> --- | 1,5-9 | A 63 F 3/06 <br> A 63 F 3/08 <br> G 07 F 7/00 <br> G 07 F 17/32 |
| Y | WO-A-8 902 139 (ENTENMANN et al.) <br> * Page 3, lines 16-24; figure 1 * <br> --- | 1,5-9 | |
| A | US-A-4 764 666 (BERGERON) <br> * Column 6, lines 4-18 * <br> --- | 1-3 | |
| A | GB-A-2 180 460 (DE PAULA MARTINEZ LOPEZ) <br> * Page 2, lines 3-19 * <br> --- | 1-3 | |
| A | GB-A-2 166 961 (MARTIN) <br> * Page 2, lines 73-75 * <br> --- | 7 | |
| A | WO-A-8 604 826 (ACORN INDUSTRIES, INC.) <br> * Page 4, lines 15,16; page 8, lines 30-34; page 11, lines 9,10; figures 1,2 * <br> ----- | 6,8 | **TECHNICAL FIELDS SEARCHED (Int. Cl.5)** <br><br> A 63 F <br> G 07 F |

## INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with
the provisions of the European Patent Convention to such an extent that it is not possible to carry
out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely : 1-9

Claims searched incompletely :

Claims not searched : 10,11

Reason for the limitation of the search:

Method for playing a game EPC art 52(2)(C)

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 17-03-1992 | GLAS J. |

EPO FORM 1503 03 82 (P0407)

# EUROPEAN PATENT APPLICATION

(12)

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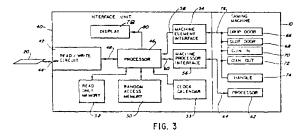**Sparks Nevada 89431 (US)**

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(54) **Game machine data transfer system.**

(57) A system of data transfer is provided for use in a game machine environment such as a casino in which a number of game machines are provided and players are free to move from one to another. Each player carries a smart card or data transfer unit (20) containing a memory (26) and a processor (22) having addressing, control, protection and/or encryption circuitry. An interface unit (40) (FIG.3) associated with each game machine (10). is adapted to receive the card (20) for accessing the data in the card memory (26). A central data processor (82) also is adapted to receive the card (20), and has means to access the data. The processor (22) is organized to record machine data from the game machine (10) in the data memory (26) of the card (20) and to modify player information in the data memory. The memory (26) may store data regarding cash flow. security violations. machine malfunctions and/or volume of play attributable to an individual player. Machine performance may be monitored and the eligibility of a player to receive premiums as a play incentive may be determined.

When the cards (20) are returned to the central data processor (82) they provide casino management a detailed overview of the casino organization and permit much statistical analysis of player and machine behaviour. The cards can be used as a means of paying winnings to players, with the players returning the cards to the central data processor (82) at a cashier's station to redeem their winnings.

FIG. 3

## Description

## GAME MACHINE DATA TRANSFER SYSTEM

Technical Field

The invention relates to the field of coin-operated amusement and gaming machine systems and in particular to systems for transferring data to and from such game machines.

Background

For several years, the automation of accounting and security functions having to do with coin-operated gaming machines has been shown to be a desirable and cost-effective method of obtaining accurate and timely data concerning the operation of these machines. In recent years, the automated accumulation of data concerning individual players, either in conjunction with the accounting and security information or without it, has also shown itself to be desirable as an effective marketing technique. Treating players in much the same manner as the airlines treat members of their "frequent flyer" clubs, game machine proprietors can, by keeping an accounting of the amount a player spends in their establishment, reward the patron accordingly. Also, by enrolling the player as a "preferred customer," the establishment can obtain a name and address, as well as certain other biographical and demographic data which is useful in the maintaining of a mailing list and other marketing efforts. There are also instances wherein the use of a player-carried device such as a magnetic-stripe card such as the card shown in U. S. patent 4,575,622 can be used to enable players to use coin-operated game devices by paying a lump sum in lieu of using individual coins. In these cases, the card is the means of identifying the player, while the actual accounting of play takes place in a central computer electronically connected to the gaming machines.

Another important function of gaming machine data transfer systems is to provide accounting and security information to casino operators. Most of these applications have in the past required the gaming machines to be directly connected to the central computer. Examples of such gaming machine data transfer systems are provided in U. S. patents 4,072,930, 4,283,709 and 4,636,951. This usually entails a large computer equipment installation, and the installation of an extensive network of data communication cables and associated equipment. And, since the central computer is usually connected to a large number of gaming machines, in some cases up to 2000 or more machines in a real time data communication configuration, a powerful and hence expensive central computer is required. Another disadvantage of these systems arises from the fact that the distance that the data can be transmitted is usually, as a practical matter, limited, thus the central computer is usually restricted to the same premises as the gaming machines. As a result, these electronic gaming machine information systems tend to be limited to gaming operations where a relatively large number of machines are located in close proximity to each other.

One approach to solving these problems was attempted in the SDS V system developed by Bally Systems Division of Bally Manufacturing Corporation in which a portable data recording unit having a microprocessor and limited semiconductor memory was used to collect data from slot machines. The data thus collected by an employee of the machine owner was transferred to a central data system when the recording unit was connected to a data input device in the central system. However this approach suffered from a number of deficiencies including limitations in the type and amounts of data that could be collected, no capability to collect player information and no ability to transfer information to the slot machine.

The Invention

The invention provides a data transfer system for use with a plurality of game machines comprising a plurality of portable data units for carrying by players, each portable data unit comprising a data memory containing player account information relevant to its associated player; an interface unit incorporated into each game machine for receiving the portable data unit carried by a player and for reading from the data memory of that portable data unit player information; and a central data processor for receiving the portable data unit carried by a player and reading therefrom the player information, CHARACTERISED IN THAT each interface unit includes means for transmitting to the data memory of a portable data unit received therein machine data; each portable data unit or each interface unit further comprises a processor for recording machine data in the data memory of a portable data unit received in an interface unit of a game machine and for modifying player information stored in the said data memory in response to selected machine data transmitted to the portable data unit by the interface unit in which it is received; and the central data processor further includes means for reading from a portable data unit inserted therein machine data relating to the game machines with which the portable data unit has been used.

The processor may be associated with either the portable data unit or the interface unit, but preferably each such unit has its own processor, the processors cooperating in the transfer of data to and from the portable data units, the game machines and the central data processor and modifying the player information stored in the data memories of the portable data units in response to the selected machine data information transmitted to the portable data units from the interface units to which they are connected.

The game machines may be gambling machines, otherwise known as gaming machines, which pay out winnings to successful players; or they may be amusement machines such as video games. In the

former case the winnings may be paid out in the usual way in coins by the machine or may be simply recorded on the portable data unit for encashment later when the portable data unit is presented to a cashier who inserts it into the central data processor to read the information stored thereon.

The exchange of information between the portable data units and the game machines via their interfaces according to the invention is essentially a two-way exchange. The game machine, through its interface, reads from the portable data unit player information including, preferably, player account information. It writes to the portable data unit machine information and modifies the player information so that when the portable data unit is subsequently presented to the central data processor the processor can obtain a range of information concerning player activity and game machine performance.

Each player or employee may thus carry a portable data unit that will permit the transmittal of player information and game machine information between game machines and a central data system. The data unit may also contain player account and win information. Machine identification and performance information can be transmitted and stored in the central data processor.

The portable data unit is typically a smart card, which is a device generally the shape and size of a standard credit card, which contains solid-state memory, as well as circuitry to enable the memory to be written to, read from and otherwise manipulated. Examples of such devices are disclosed in U.S. Patents 4,675,516, 4,725,924, 4,727,246, 4,733,061 and 4,764,666.

A very significant advantage of the invention is that a large central computer can be replaced by a minicomputer, or in some cases by one or more desktop personal computers. Since the need for real-time on-line on-line communication with all the game machines does not exist, the data processing functions can be handled easily by the small computer system. Since the communication of data from the game machines to the computer system is not limited by a network of data communication cables, game machines need not to be on the same premises as, or even in the near vicinity of, the computer.

The elimination of the data communication hardware and the large central computer makes possible the automation of the accounting. security and player tracking even for small establishments, and to operators that have small numbers or widely dispersed gaming machines which could not otherwise justify the cost of such a system.

Drawings

FIG. 1 is a perspective view of a gaming machine;
FIG. 2 is a partially cut away perspective view of a portable data transfer unit being part of a system according to the invention;
FIG. 3 is a functional block diagram of a gaming machine interface circuit for use with

the data transfer unit of FIG. 2, along with a portion of the gaming machine circuitry;
FIG. 4 is a functional block diagram of a central data processing system for use with the data transfer unit of FIG. 2;
FIG. 5 is a diagram of memory organization for a player data transfer unit as in FIG. 2;
FIG. 6 is a diagram of memory organization for a machine data transfer unit as in FIG. 2; and
FIG. 7 is a diagram of a down load data transfer unit as in FIG. 2.

Best Mode

In FIG. 1 is provided a view of a typical gaming machine 10. The machine 10 as represented in FIG. 1 is a slot machine which in normal operation accepts a coin or token in a slot 12 and responds to a pull on a handle 14 by a player by depositing one or more coins in a coin tray 16 if a winning play is registered. A win or lose indication is provided to the player by a display 18 that in a slot machine normally takes the form of a group of mechanical reels or a video display. Although the gaming machine 10 shown in FIG. 1 is a slot machine, it should be understood that the invention can be equally applied to a wide variety of casino-type gaming machines including video poker and 21 machines as well as other coin operated amusement games.

FIG. 2 provides a partially cut away view of the preferred embodiment of a portable data transfer unit 20. The data unit 20 includes a microprocessor 22 connected by data lines 24 to a number of non-volatile random access semiconductor memories 26. Microprocessor 22 is connected to an interface circuit 28 by a group of data and control lines indicated generally at 30. Communication to external devices is facilitated by a group of contacts 32 connected to the interface circuit 28. Preferably, these contacts conform to an industry standard such as ISO/DIS 78161/1 and 78161/2 and include contacts for: chip select signals; clock input signals; a data input signal; a data output signal; a power supply input; a status input signal, a ground line and a memory type signal.

The circuit elements 22, 26 and 28 can in effect function as a small computer system by, for example: accepting data and control signals from external devices connected to contacts 32; using the microprocessor 22 to process the data; reading and writing data into memory 26 and transmitting data and control signals via the interface circuit 28 to the external devices.

In the embodiment of FIG. 2, the circuit elements 22, 26 and 28 are mounted on a bottom sheet 34 and covered or sealed within the unit 20 by a cover sheet 36 with a portion 38 left open to provide access to the contacts 32. Preferably the data unit 20 has outer dimensions that correspond to a standard credit card. Examples of various types of structures that can be used for the portable data unit 20 are shown in U. S. patents 4,725,924, 4,727,246, 4,733,061 and 4,764,666.

Although the preferred embodiment of the portable data unit 20 has been described above in terms of an IC card or smart card, other configurations or

structures that provide a data memory along with a data processing capability arranged in a conveniently portable package could serve as well.

Use of the data unit 20 with the gaming machine 10 is facilitated by an interface unit 40 secured within the housing of the gaming machine 10 as shown in FIG. 3. Although the preferred embodiment of the invention in FIG. 1 shows the interface unit 40 secured within the machine 10, the machine 10 can also include the interface unit 40 by attaching it as a separate unit to the exterior of the machine's 10 housing. A read/write unit 42 receives the data unit 20 via a slot 44 configured in the housing of the gaming machine 10. The read/write circuit 42 contains contacts corresponding to the contacts 32 of the data unit 20 along with conventional signal interface and buffering circuitry. Additionally included in the interface unit is a microprocessor 46 connected to the read/write unit 42 by data and control lines represented by a line 48 and a random access memory 50 along with a read only memory 52 that contains control instructions for the processor 46. A clock-calendar circuit 53 is also connected to the processor 46.

Since the preferred embodiment of the invention is described within the context of a microprocessor based slot machine 10, the interface unit 40 also includes a machine element interface circuit 54 and a machine processor interface circuit 56 connected to the processor 46 by data and control lines represented by lines 58 and 60 respectively.

The slot machine 10 includes, as shown in FIG. 3, a microprocessor 62 that communicates with the machine processor interface 56 via a data bus 64. As is conventional with microprocessor based gaming machines including video poker and other types of gaming machines, the microprocessor 62 controls the operation of the machine 10. Slot machines of the type shown in FIG. 3 also have a number of discrete signal sources that represent the status of the machine. Representative examples of status signals generated in the slot machine 10 are: drop door status 66, slot door status 68, a coin in signal 70, a coin out signal 72 and a handle pull signal 74. The signal sources 66-74 are connected to the machine element interface 54 by a series of lines represented for simplicity by a single line 76.

The preferred embodiment of the interface unit 40 additionally includes a display 78 which is connected to the microprocessor 46 by a conventional set of data, address and control lines as represented by a line 80.

Also, as illustrated in FIG. 4, the data transfer system includes a central data processor 82 to perform general casino accounting functions and which receives the data unit 20 in a system interface circuit 84. The interface circuit 84 includes contacts and a read/write circuit (not shown) which is similar in operation to the read/write circuit 42 of FIG. 3. The system of FIG. 4 can also have one or more data input terminals 86 and displays 88. Depending upon circumstances such as the number and locations of gaming machines 10 and the functions to be performed by the central data processing system of FIG. 4, the preferred embodiment of the hardware

arrangement will vary. For example, in some instances where a large number of machines 10 are being administered in a centralized accounting system the central data processor 82 would be a minicomputer with one or more terminals 86 directly connected to the computer 82 in the same location. On the other hand, where a fewer number of gaming machines 10 are to be administered or a more decentralized accounting system is used, one or more personal computers can be used for the data processor 82, terminal 86 and display 88. Where it is desired to use more than one personal computer to perform the functions of the system of FIG. 4, and it is considered important to maintain centralized control of the gaming data and transactions, a data communication network such as a local area network can be used to operatively tie the computers 82 together.

In any event, the system as illustrated in FIGS. 1-4 provides substantially enhanced flexibility along with increased operational capabilities in a gaming machine data and control system while at the same time reduces hardware requirements and costs.

Operation of the system of FIGS. 1-4 will now be described in connection with the memory diagrams of FIGS. 5-7. With reference to FIG. 5 which illustrates the data organization in the memory 26 in a first embodiment 90 of the portable data unit 20 of FIG. 2 for use by a player, the data unit or card 20 at the time that it is first issued to a player by a casino or game operator is inserted into the system interface 84 of the central system of FIG. 4. At this point the various data fields in the memory 90 are initialized.

A first field 92 is loaded with data indicating the type of card and an identification number for that particular card 20. As will be described below, there are a number of different types of cards 20 that can be employed by this system. At this time data identifying the player is also written into a data field 94. This information is particularly important due to the fact that many casinos base their marketing programs around the amount of activity of individual players or customers and as such it is very useful to be able to track the amount of play activity of each player. In this manner, for example, premiums and bonuses can be awarded to particularly active players thereby encouraging further play on the part of the individual. In addition, this information can be used to compile lists of preferred customers which is useful from a marketing standpoint.

Another data field 96 can be used to store casino or card data such as an identification of the casino, the location of the issuing system, player account number and the number of times that the particular card 20 has been used.

In the event that the card 20 is to be used as a debit or a credit card to play the gaming machines 10, that information is loaded into a debit/credit field 98 by a casino employee using the terminal 86. If the card 20 is used as a debit card the employee accepts payment and loads the appropriate monetary value in the field 98 or if the card 20 is to be used as a credit card, the employee authorizes the amount of credit that the particular player is entitled to and

loads that amount into field 98. By the same token, the information in the field 98 can be used by the casino employee using terminal 86 to pay or credit the player when the card is redeemed or presented for payment.

Since security is an extremely important consideration in casino operations, the memory 90 can also include an encryption data field 100 which can contain encryption keys or algorithms. As will be described in more detail later, the data in field 100 can be used by the interface unit 40 to ensure that the card 20 is an authorized card. Also the data in the other fields of the memory 90 can be encrypted such as the debit/credit field 98 in order for example to discourage players from taking the card 20 home to increase on their own the value of the credits in that field

After the player card 20 having memory organization 90 is initialized, it is ready for use with the gaming machine 10. The player will insert the card 20 into the read/write circuit 42 of the interface unit 40 as shown in FIG. 3 via slot 44. The read/write circuit 42 is under the control of the microprocessor 46 and causes this circuit 46 to transmit to the microprocessor 22 on the card 20 the appropriate instructions so that the identifying data in fields 92 and 94 are transmitted to the processor 46. Depending upon the level of security desired the processor can also access encryption keys or passwords from field 100 to use with an encryption algorithm or password procedure stored for example in ROM 52 to determine if the card 20 is authorized. The information stored in field 100 can additionally include access control codes and codes defining the functions and access capabilities of the card 20. Alternatively the card microprocessor 22 can encrypt using, for example, one of the NBS encryption standards the data being transmitted to the processor 46 and the processor 46 can in turn use a matching algorithm to decrypt the data.

Once the card 20 has established communication with the interface unit 40 and been identified as a player type card, the interface unit 40 will make available to the gaming machine 10 the debit or credit information contained in card field 98. In the embodiment shown in FIG. 3, this information is transmitted by way of the machine interface 56 to the gaming machine microprocessor 62. In gaming machines of this type the player has the option of selecting by means of a control button or switch indicated at 101 on the machine housing 10, credit or coin operation.

One of the more significant features of the invention is a comprehensive player tracking capability As the player operates the machine, data representing game play is transmitted by the interface unit to the memory 90 of the card 20. For example, the identification of the machine being played is stored in a data field 104 as shown in FIG. 5. In the preferred embodiment of the invention the identification of the last ten machines 10 played are stored in fields 104. In addition, specific information relating to the games played is also stored in card memory 90. In the embodiment shown in FIG. 5, eight data fields indicated generally at 106 are

provided to store information relating to player activity. Here, there is one field 106 for each denomination: nickel, dime, quarter, half-dollar, dollar, $5, $25 and $100. Within each field 106 there is a group of subfields for storing the number of: coins played 108, coins paid out 110, the number of games played 112 and the number of coins paid by attendants 114 for each denomination. Also, the time of play in minutes for that denomination is stored in a subfield 116. It will be understood, of course, that the amount and types of data stored in the game play fields such as 106 of memory 90 can be varied to suit a particular casino operating environment. In addition to the play data discussed above the memory 90 contains a data field 118 to store information relating to the jackpots or other major prizes won by the players.

In the preferred embodiment of the invention, the interface unit 40 performs the calculations necessary to compute the players bonus or premium award status and stores that status in a bonus data field 102 in the card memory 90. The bonus status can be based on a wide variety of information and criteria such as the number of games or time the machine 10 is played as received from the machine processor 62 along with other data obtained from the card memory 90. For example, player bonus status can be calculated on the player's "volume of play" which can include one or more of the following factors: coins played, number of handle pulls, amount won, length of time played, payouts, length of time played without a win, etc. Use of the processor 46 in the interface unit 40 to perform this function has a number of advantages including immediate access to relevant information about the individual player and a complete operational separation and non-interference with the operation of the gaming machine 10.

The use of the card or data transfer unit 20 enhances the data gathering ability of the gaming machine data transfer system when the player redeems the card 20. Typically the player will hand the card 20 to a casino employee who will insert the card 20 into the system interface 84 of the central data system of FIG. 4. Upon receiving and verifying the data from card 20 the central data processor 82 can, either automatically or at the request of an employee operating the terminal 86, clear selected portions of memory 90, thus preparing the card 20 for future data collection. The system of FIG. 4 can also display on display 88 or print out reports on information and calculations based on the data thus collected. At this point the player, based on the data displayed, can receive payment or credit derived from the information on the card 20. In particular, the player's individual account status is printed out or displayed on display 88 so that the casino employee can determine what prizes, premiums or awards that the player may be entitled to. If such awards are made by the employee, this information is entered into the system of FIG. 4 via the terminal 86 resulting in a decrementing of the bonus data in field 102 of card memory 90. As with the interface unit 40, it is the system interface 84, which is functionally similar in construction to interface 40, that actually performs

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the reading from, writing to and clearing of card memory 90.

Cashless gaming is also facilitated by use of the card 20 in combination with the system of FIGS. 3 and 4. When the player is issued a card 20 or presents a previously issued card to a casino employee, the employee will insert the card 20 into the system interface 84 and by using the terminal 86 updates the debit or credit balance in field 98 of card memory 90. Upon receipt of the card 20 in interface unit 40, the processor 46 first verifies that there is sufficient value in field 98 to play the machine 10 and then via machine interface 56 enables the machine 10 for credit play. The player by utilizing the machine control 102 can select normal payouts via the coin tray 16 or to have the balance on field 98 reflect his winnings. Win data if the option is selected, is transmitted from the machine processor 62 through the interface unit to data field 98. The credit or debit status, which can be termed account status, as reflected in field 98 is provided to the player by the display 78 which is controlled by the processor 46.

A related feature of the interface unit 40 is the ability to provide information on the display 78 in addition to the player's debit or credit balance. For example, using the data stored in memory 90, the processor 46 can display the player's name in a personalized welcoming message along with other player specific information such as any bonuses or prizes he may be entitled to as indicated in data field 102.

The use of the portable data transfer unit 20 has a number of very significant advantages in collecting data regarding gaming machine operation and security as well. As shown in FIG. 6, a portion 120 of the card memory 26 can be configured to collect data relating to machine operation. Although the memory 120 as shown in FIG. 6 is structured for a particular type of card 20, it should be understood that the data organization could be incorporated into other types of cards such as the player card 20 of FIG. 5. For purposes of this description the data fields in FIGS. 5-7 that contain similar types of data will have the same reference numerals.

With respect to FIG. 6, if the card 20 containing memory 120 is an employee issued card data field 92 will reflect that fact and data field 94 will contain the employee's identification. In the embodiment of the memory organization 120, shown in FIG. 6, there are a group of data fields indicated at 122 each of which corresponds to a particular gaming machine. The data fields 122 are each divided into a number of subfields. When the card 20 is inserted into the interface unit 40 the processor 46 will write the gaming machine 10 identification in a first subfield 124. During normal operation of the gaming machine 10 accounting and security data is transmitted via the machine element interface 54 and the processor interface 56 to the interface processor 46 and stored in the memory 50. Preferably this data is time and date stamped by the processor 46. After the card 20 has been verified by processor 46 and the machine identification stored in the subfield 124, the processor 46 will write machine accounting information such as: the number of coins played into a subfield

126; the number of coins in the gaming machine 10 cash box into a subfield 128; the number of coins paid out by the machine 10 into a subfield 130; the number of games played for card-type gaming machines or handle pulls for slot type gaming machines into a subfield 132 and the number of coins paid by attendants to players into a subfield 134. Similarly, security information such as the number of gaming machine door openings will be written into a subfield 136; the number of coin hopper jams will be written into a subfield 138 and the number of blackouts, that is interruptions of electrical power to the machine 10, will be written into a subfield 140. Also, the last ten security events such as tilts and illegal pays recorded in the memory 50 will be written into a subfield 142. Along with the data as described above appropriate date-time information corresponding to the data will be written into the subfields 126-142 as well. It should be noted that the use of a separate processor 46 and memory 50 in the interface unit 40 makes possible a particular convenient method of gathering and retaining time information which can be very useful from a security standpoint. As with the player data unit 90, the employee card having memory organization 120 would be inserted into the system interface 84 and the machine data transferred from the card 20 to the central data processor 84.

As indicated above, the memory 26 of the data transfer unit 20 can store various types of player and machine data. For example, it would be possible to include portions of the data organization 120 in the data organization 90 of the player card 20. In this manner the players would collect at least a portion of the machine information of FIG. 6 in the course of playing the gaming machine 10. This could reduce the need for casino employees to make regular checks of the status and performance of the gaming machines and provide more current information with respect to at least the more heavily patronized machines.

A third type of data organization 144 for use with the data transfer unit 20 is illustrated in FIG. 7. The cards having this type of data would be used by casino employees for down loading control programs and data to the gaming machine 10 and interface unit 40. Examples of such control instructions and data include control instructions for the interface unit processor 46 stored in a field 146 and player messages for use with the display 78 stored in a field 148. Updates for the interface units 40 encryption/decryption keys and passwords can be stored in a data field 150 and revised encryption algorithms for use by the processor 46 can be stored in a data field 152. A data field 154 can contain new or revised computer programs for controlling the gaming machine processor 62 along with control variables that the processor 62 employs, for example, in setting the win percentage of the gaming machine 10. The data in memory 144 can also include a field 156 storing formulas used by the interface unit processor 46 to calculate the player bonus or premiums.

Also shown in FIG. 7 is a field 158 for storing card use data. This data is written into field 158 by the

interface unit 40 each time the card 20 is used with a gaming machine l0 and as such provides the central data system of FIG. 4 and hence casino management with valuable information regarding card usage. Although not shown in FIGS. 5 and 6 it is considered desirable in the preferred embodiment of the invention to include a similar data field in the player card memory 90 and the machine data memory 120.

From the above discussion it is apparent that the use of the portable data unit or card 20 having a data processing capability in combination with computer controlled gaming machine interface units 40 provides a gaming machine information transfer system of great flexibility and low cost with the ability to generate useful information for casino management. Although the invention has been described in terms of its use with gaming machines that return money to the player, such as slot machines or video poker machines, many of the aspects of the invention would apply to coin-operated amusement type games as well. Thus, certain aspects of the invention relate to game machines in general which include gaming machines along with amusement games.

**Claims**

1. A data transfer system for use with a plurality of game machines (10) comprising:
a plurality of portable data units (20) for carrying by players, each portable data unit (20) comprising a data memory (26) containing player account information relevant to its associated player;
an interface unit (40) incorporated into each game machine (10) for receiving the portable data unit (20) carried by a player and for reading from the data memory (26) of that portable data unit (20) player information; and
a central data processor (82) for receiving the portable data unit (20) carried by a player and reading therefrom the player information, CHARACTERISED IN THAT each interface unit (40) includes means for transmitting to the data memory (26) of a portable data unit (20) received therein machine data; each portable data unit (20) or each interface unit (40) further comprises a processor (22 or 46) for recording machine data in the data memory (26) of a portable data unit (20) received In an interface unit (40) of a game machine (10) and for modifying player information stored in the said data memory (26) in response to selected machine data transmitted to the portable data unit (20) by the interface unit (40) in which it is received: and
the central data processor (82) further includes means for reading from a portable data unit (20) inserted therein machine data relating to the game machines (10) with which the portable data unit (20) has been used.

2. A system according to claim 1, wherein each portable data unit (20) comprises a processor (22) for modifying the player informa-

tion stored in the data memory (26) of the portable data unit (20) when received in an interface unit (40) in response to the selected machine data transmitted to the portable data unit (20) by the interface unit (40).

3. A system according to claim 1, wherein each interface unit (4o) comprises a processor (46) for modifying the player information stored in the data memory (26) of a portable data unit (20) inserted therein, in response to the selected machine data transmitted to the portable data unit (zo) from the interface unit (40).

4. A system according to claim 1, wherein each portable data unit (20) comprises a processor (22) and each interface unit (40) comprises a processor (46), the processors (22, 46) cooperating to modify the player information stored in the data memory (26) of a portable data unit (20) received in an interface unit (40) in response to the selected machine data transmitted to the portable data unit (20) by the interface unit (40).

5. A system according to any preceding claim, in which the data memory (26) is a non-volatile random access memory.

6. A system according to any preceding claim, in which the interface units (40) are included within the outer housings of the game machines (10).

7. A system according to any preceding claim, in which both the interface units (40) and the central data processor (82) comprise means for encrypting data transmitted to the portable data unit (20) and means for decrypting data transmitted from the portable data units (20).

8. A system according to any preceding claim, in which each interface unit (40) includes means for adding date and time information to the selected data.

9. A system according to any preceding claim, in which each interface unit (40) additionally includes a display (78) for displaying player information from a portable data unit (20) inserted therein.

10. A system according to any preceding claim, in which each portable data unit (20) has outer dimensions that correspond to a standard credit card.

11. A system according to any preceding claim, wherein each portable data unit (20) has a separate memory or memory area (26) configured to receive player information, play information and machine data.

12. A system according to claim 11, wherein the play information includes player account information including separate fields to identify, for each denomination of currency acceptable to the game machine, the number of games played, the amount of any winnings, the number of coins or tokens inserted into the game machine while the portable data unit (20) is inserted therein and the time played.
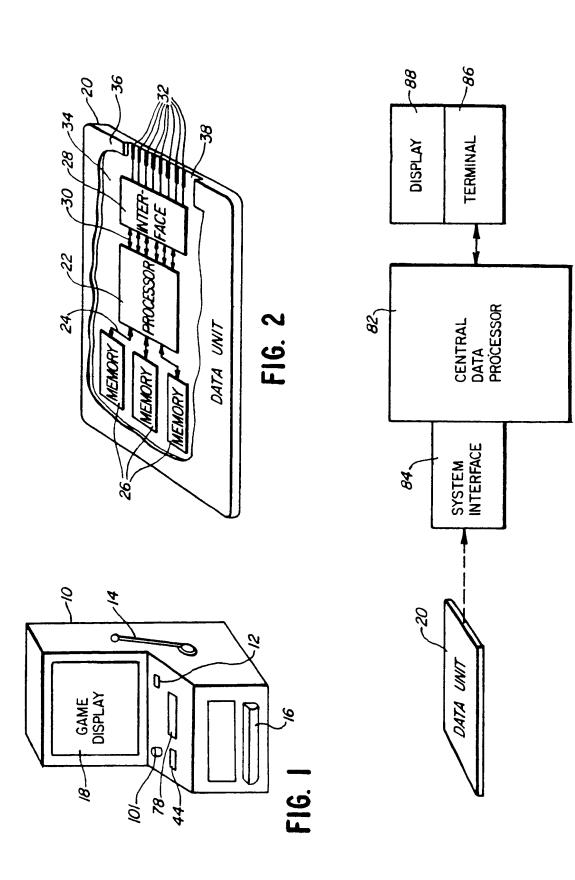
FIG. 2



FIG. 4



FIG. 1

FIG. 3

**FIG. 7**

DOWNLOAD DATA — 144

- CARD ID/TYPE — 92
- EMPLOYEE ID — 94
- CASINO DATA — 96
- INTERFACE UNIT CONTROL INSTRUCTIONS — 146
- PLAYER DISPLAY MESSAGES — 148
- ENCRYPTION / DECRYPTION KEYS — 150
- ENCRYPTION / DECRYPTION ALGORITHMS — 152
- MACHINE DATA / INSTRUCTIONS — 154
- PLAYER BONUS FORMULAS — 156
- CARD USE DATA — 158
- ENCRYPTION DATA — 110

**FIG. 6**

MACHINE DATA — 120

- CARD ID/TYPE — 92
- PLAYER / EMPLOYEE ID — 94
- CASINO DATA — 96
- MACHINE ID — 124
- COINS PLAYED — 126
- COINS TO CASH BOX — 128
- COINS PAID OUT — 130
- GAMES PLAYED — 132
- COINS BY ATT. — 134
- # OF DOOR OPENINGS — 136
- # OF COIN JAMS — 138
- # OF BLACKOUTS — 140
- SECURITY EVENTS (LAST 10) — 142

— 122

- MACHINE ID — 124
- SECURITY EVENTS (LAST 10) — 142
- ENCRYPTION DATA — 100

**FIG. 5**

PLAYER DATA UNIT — 90

- CARD ID/TYPE — 92
- PLAYER NAME / ID — 94
- CASINO DATA — 96
- MACHINE PLAYED
- MACHINE PLAYED — 104
- MACHINE PLAYED

PLAY INFORMATION

- COINS PLAYED — 108
- COINS PAID — 110
- 5¢
- GAMES PLAYED — 112
- COINS BY ATT. — 114
- TIME PLAYED — 116

$100

- COINS PLAYED
- COINS PAID
- GAMES PLAYED
- COINS BY ATT
- TIME PLAYED

— 106

- DEBITS / CREDITS — 98
- JACKPOTS — 118
- ENCRYPTION DATA — 100
- BONUS DATA — 102

# EUROPEAN PATENT APPLICATION

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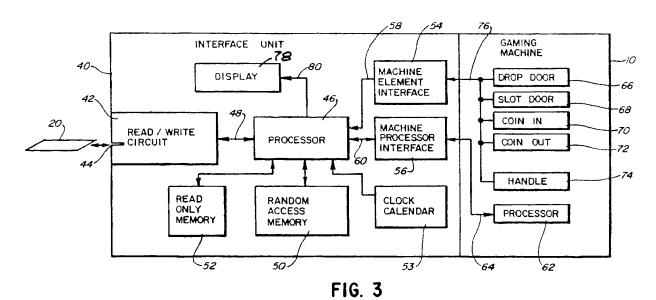**444 Solaga Ct.**
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(54) Game machine data transfer system.

(57) A system of data transfer is provided for use in a game machine environment such as a casino in which a number of game machines are provided and players are free to move from one to another. Each player carries a smart card or data transfer unit (20) containing a memory (26) and a processor (22) having addressing, control, protection and/or encryption circuitry. An interface unit (40) (FIG.3) associated with each game machine (10). is adapted to receive the card (20) for accessing the data in the card memory (26). A central data processor (82) also is adapted to receive the card (20), and has means to access the data. The processor (22) is organized to record machine data from the game machine (10) in the data memory (26) of the card (20) and to modify player information in the data memory. The memory (26) may store data regarding cash flow. security violations, machine malfunctions and/or volume of play attributable to an individual player. Machine performance may be monitored and the eligibility of a player to receive premiums as a play incentive may be determined.

When the cards (20) are returned to the central data processor (82) they provide casino management a detailed overview of the casino organization and permit much statistical analysis of player and machine behaviour. The cards can be used as a means of paying winnings to players, with the players returning the cards to the central data processor (82) at a cashier's station to redeem their winnings.

EP 0 360 613 A3

**FIG. 3**

European
Patent Office

EUROPEAN SEARCH
REPORT

Application Number

EP 89 30 9659

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| D,X,A,Y | US-A-4 575 622   (PELLEGRINI)<br>" the whole document " | 1,2,3,4,6,<br>10-12,<br>2-5,8-12 | G<br>07 F 17/34<br>G 07 F 17/32 |
| D,Y,A | US-A-4 764 666   (BERGERON)<br>" the whole document " | 2-5,8-12,<br>1 | |
| X,A | DE-A-3 441 518   (GAUSELMANN)<br>" abstract " " page 6 " | 1,8,10,<br>2-4,11 | |
| P,A | DE-A-3 802 186   (NSM-APPARATEBAU)<br>" the whole document " | 1-12 | |
| A | GB-A-2 161 629   (JOHN ARNOLD KLAYH)<br>" abstract " | 1 | |
| | | | **TECHNICAL FIELDS SEARCHED (Int. Cl.5)** |
| | | | G 07 F |

The present search report has been drawn up for all claims

| Place of search | Date of completion of search | Examiner |
|---|---|---|
| The Hague | 19 March 91 | TACCOEN J-F.P.L. |

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another
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O : non-written disclosure
P : intermediate document
T : theory or principle underlying the invention

E   earlier patent document, but published on, or after
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D   document cited in the application
L   document cited for other reasons
............................................................................
&   member of the same patent family, corresponding
    document

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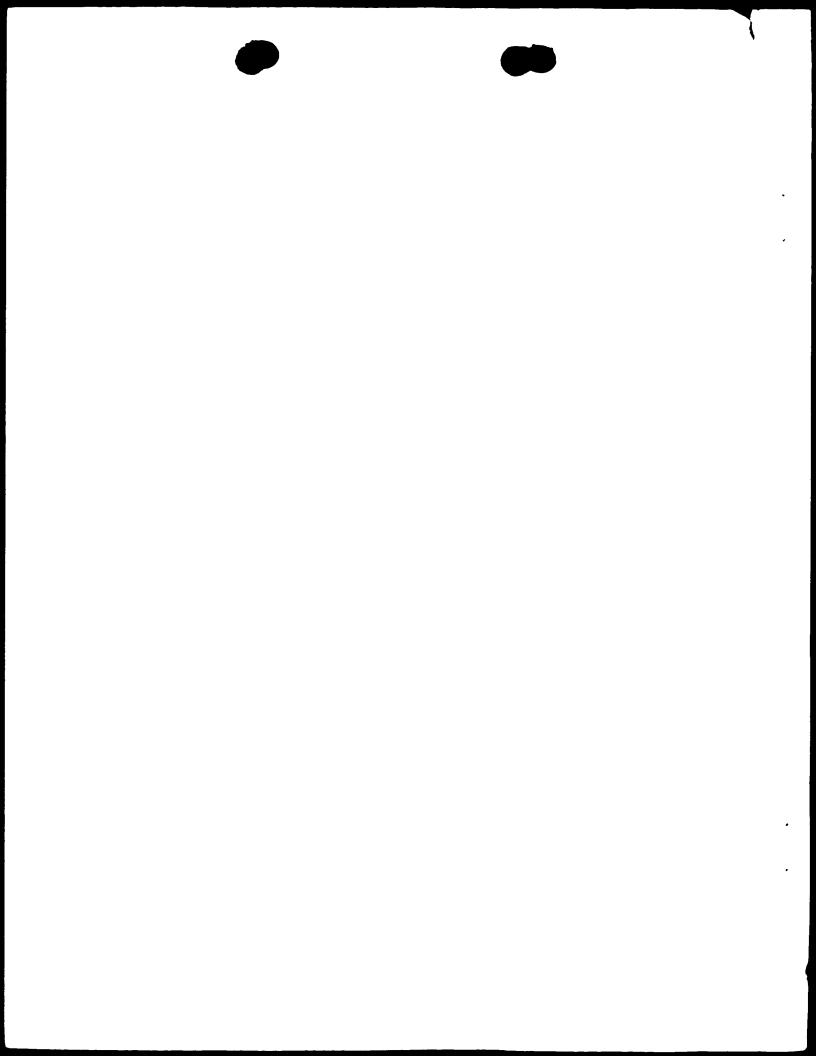
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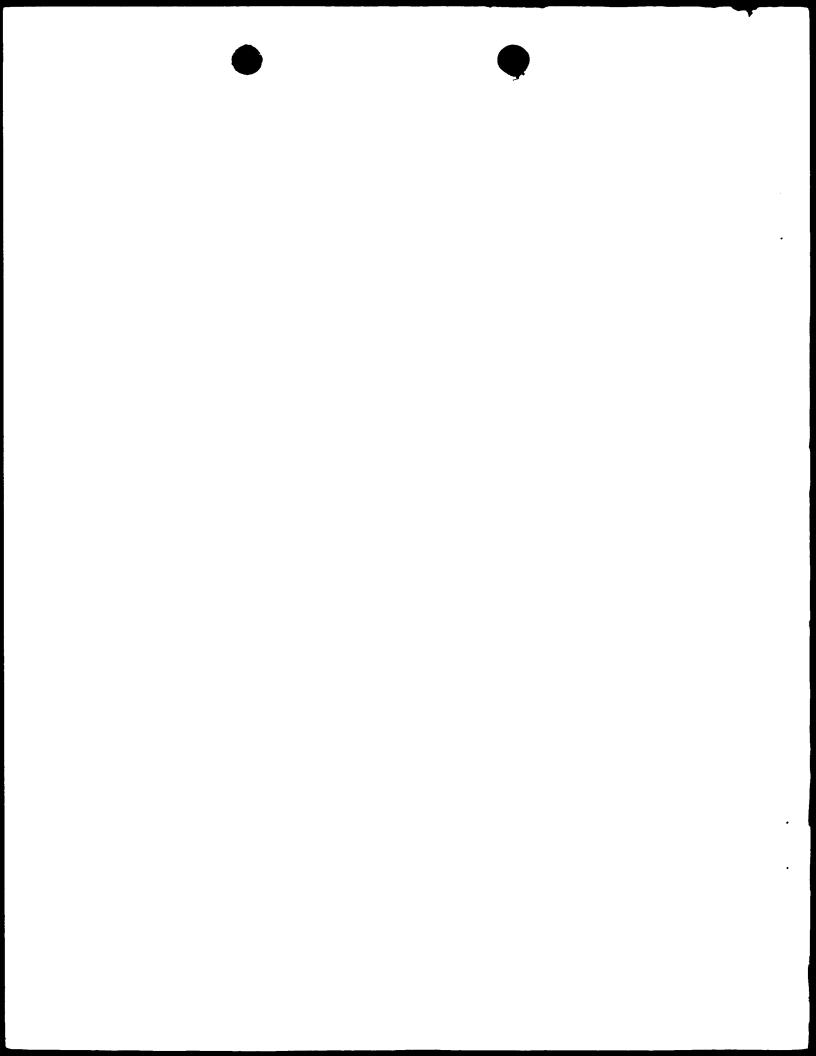
(57) Abstract

The present invention relates to tumor associated antigen (TAA) peptides and to the use of same, of polynucleotides encoding same and of cells presenting same as anti–tumor vaccines. More particularly, the present invention relates to tumor associated antigen peptides derived from Uroplakin Ia, Ib, II and III, Prostate specific antigen (PSA), Prostate acid phosphatase (PAP) and Prostate specific membrane antigen (PSMA), BA–46 (Lactadherin), Mucin (MUC–1), and Teratocarcinoma–derived growth factor (CRIPTO–1) and the use of same as anti–tumor vaccines to prevent or cure bladder, prostate, breast or other cancers, carcinomas in particular. Most particularly, the present invention relates to tumor associated antigen peptides which are presentable to the immune system by HLA–A2 molecules.

# TUMOR ASSOCIATED ANTIGEN PEPTIDES AND USE OF SAME AS ANTI-TUMOR VACCINES
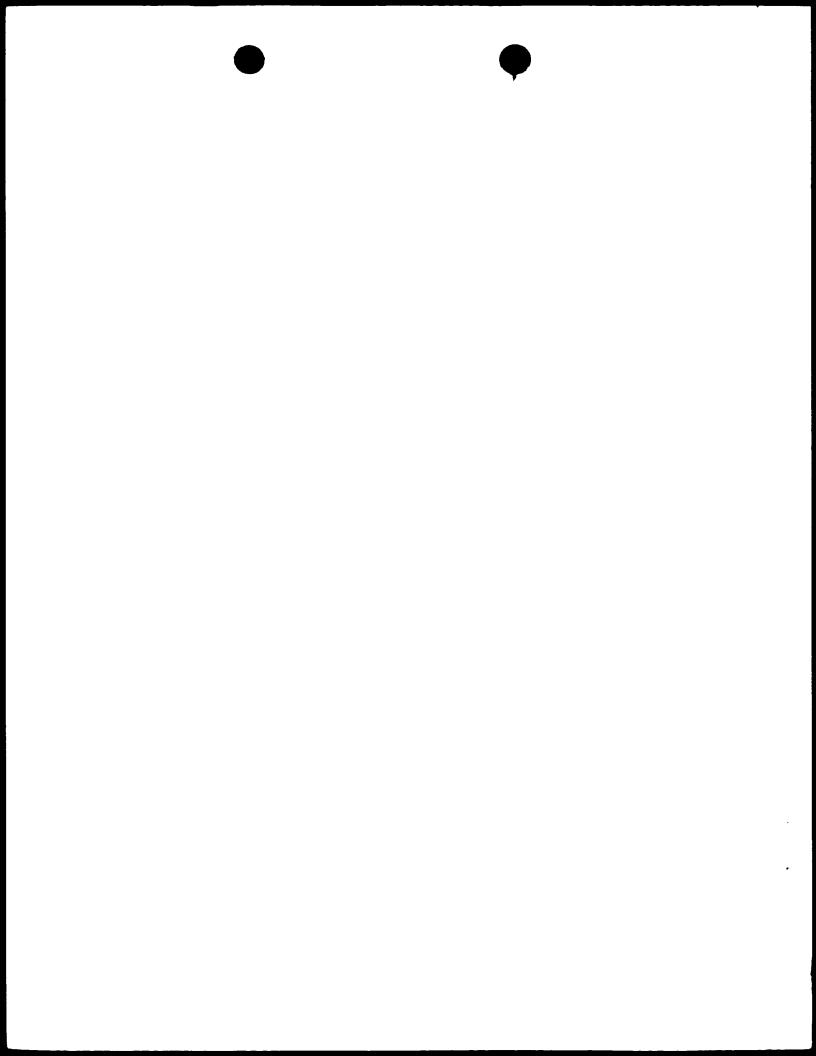
## FIELD AND BACKGROUND OF THE INVENTION

5      The present invention relates to tumor associated antigen (TAA) peptides and to the use of same, of polynucleotides encoding same and of cells presenting same as anti-tumor vaccines. More particularly, the present invention relates to tumor associated antigen peptides derived from Uroplakin Ia, Ib, II and III, Prostate specific antigen (PSA), Prostate acid phosphatase (PAP) and Prostate

10     specific membrane antigen (PSMA), BA-46 (Lactadherin), Mucin (MUC-1), and Teratocarcinoma-derived growth factor (CRIPTO-1) and the use of same as anti-tumor vaccines to prevent or cure bladder, prostate, breast or other cancers, carcinomas in particular. Most particularly, the present invention relates to tumor associated antigen peptides which are presentable to the immune system by

15     HLA-A2 molecules.

Local therapy such as surgical excision or ablation by radiation is a mainstay for the treatment of primary cancer and is curative for a percentage of patients. However, many malignancies will recur locally or at a distant site. Thus the prevention or cure of metastases remains a major focus in clinical oncology

20     (1). Although early detection followed by surgery provides good prognosis for a number of major cancer types, a large fraction of patients would need adjuvant therapy. Part of these patients will, with time, succumb to metastasis (2-4). Alternative approaches based on gene therapy and immunotherapy have been the focus of attention in the last years. One such approach is specific active

25     immunotherapy (SAI, 5). The objective of SAI is to stimulate a tumor specific cytotoxic T lymphocytes (CTL) immune response that is capable of eliminating residual metastatic disease and induce a state of immunity to protect the patients from recurrent disease. The underlying assumption of SAI is that tumor cells express tumor antigens which are sufficiently distinct in structure or context to

30     induce an effective CTL response (6). Although the validity of these assumptions was questioned, a number of studies in the last decade have demonstrated the rational of SAI. In a landmark study, van Pel and Boon have shown that tumor associated antigens (TAAs) can be isolated and defined (7). Importantly, *ex-vivo* manipulations of "non-immunogenic" animal tumor cells can be used to elicit

35     effective immune responses which will also recognize parental "non-immunogenic" tumor cells (8). Studies employing rodent tumor models with little intrinsic immunogenicity have shown that genetically modified tumor cells transduced to express MHC class I, cytokines such as IL-1, IL-2, IL-4, IL-6, IL-7, IL-12, IFN or GM-CSF or costimulatory molecules such as B7-1 or B7-2 were
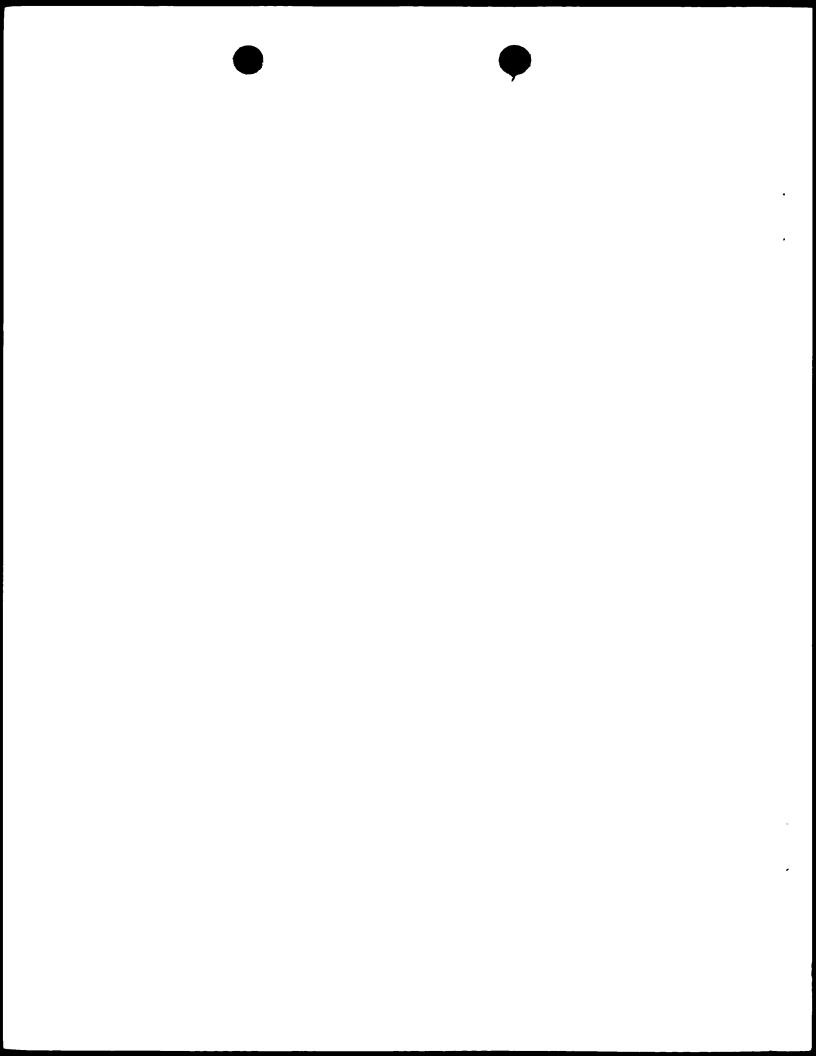
capable of preventing or causing regression of tumors or metastases (reviewed in 9). Although gene modified tumor vaccine (GMTV) clinical trials, with improved retroviral vectors or other transfer methodologies are currently tested, it becomes clear that GMTV using autologous tumor cells might be limited by its complexity, high cost and ineffective gene transfer methodologies (10). One alternative approach would be vaccination with tumor associated antigens (TAAs) presented in an effective way to the patient's immune system, to induce antigen specific CTL (11).

Cytotoxic T lymphocytes (CTL), directed against peptides presented by MHC class I molecules, constitute powerful effectors of the immune system against tumors or infectious agents (12). These peptides are usually 8-10 amino acids long with 2-3 primary anchor residues that interact with the MHC class I molecules and 2-3 amino acid residues which bind to the T cell receptor (13). Several methods have been employed to identify CTL epitopes. If the amino acid sequence of a protein antigen is known, like in the case of viral proteins, oncogenes, suppressor genes or growth factor receptors, overlapping peptides of 8-10 amino acids in length can be synthesized and screened as CTL targets (14). CTL epitopes may also be identified subsequent to the search for MHC binding motifs in known proteins (15). If the tumor antigen is not known, isolation of the TAA peptides from total acid extract or from MHC class I molecules followed by HPLC fractionation steps and Edman sequencing (16) or mass spectrometry (17) provide a direct way of identifying CTL epitopes. Recently, a synthetic combinatorial library approach, in which defined amino acids in two MHC anchor positions are fixed and all other positions are subgrouped for CTL screening has led to the description of novel EL4 TAA peptide mimotopes (18).

The most fruitful method, so far, designed by T. Boon and his colleagues is the genetic approach in which cDNA expression libraries are pool transfected into COS7 cells with the appropriate HLA and screened by CTL lines. This approach led to the discovery of several human melanoma and mouse mastocytoma antigens recognized by specific CTL (19). The first report of a phase I clinical trial with the synthetic MAGE3 melanoma peptide, restricted by HLA-A1, showed regression of cutaneous, subcutaneous and lung metastases in 3/6 patients (20). Recently, two reports of clinical trials have shown that treatment of patients with a melanoma gp100 TAA peptide together with IL-2 resulted in significant tumor regression in 13/31 (42 %) patients and that vaccination with defined peptides or total peptide extracts on autologous dendritic cells (DC) resulted in complete or partial cures (21, 22). Regression of lung carcinoma established metastases or small established tumors was demonstrated in a murine model by peptide

vaccination (23, 24). These observations suggest that TAA peptide vaccines may constitute a reasonable therapeutic modality in advanced cancer. In studies with murine tumors, CTL are induced *in vivo* by immunization with irradiated tumor cells, often gene modified by MHC class I; cytokine or costimulatory molecules
5 like B7-1 or B7-2 genes (16, 18, 25). In melanomas, CTL lines were mostly induced from peripheral blood mononuclear cells (PBMC) of patients or from tumor infiltrated lymphocytes (TIL, 19, 26). Yet, most metastatic tumors are non-immunogenic tumors and it is extremely difficult to derive CTL lines or clones from TIL or patient's PBL. Moreover, *in vitro* propagated CTL clones do
10 not always represent dominant anti-tumor specificities but rather sporadic clones surviving culture conditions. Lately, a number of studies have compared the CTL repertoire of viral or other defined peptides, restricted by HLA-A2.1 in human PBL from HLA-A2.1 expressing patients to CTL induced in HLA-A2.1 transgenic mice. Good concordance between human HLA-A2.1 and murine transgenic
15 HLA-A2.1 CTL repertoire was found, confirming the potential of such transgenics in identification of human CTL epitopes (27). Although vaccination with defined peptides of HLA transgenic mice shows an overlapping repertoire to human CTL, vaccination of such mice with multi-epitope proteins shows that murine H-2 restricted responses are dominant and obliterate, as a rule, cytolytic responses with
20 direct recognition of human HLA (28). Thus, by combining classical HLA class I transgenesis with selective destruction of murine H-2, it is possible to derive useful mouse strains for the study of HLA class I restricted responses. While reducing the present invention to practice, we utilized such mice for preparation of anti-tumor CTL as a tool for TAA purification and as a model system to assess the
25 immunogenicity of peptides.

Murine H-2 knockout mice transgenic for a single human HLA seem to be a suitable model for induction of anti-tumor CTL. Classical $\beta_2$ microglobulin knockout mice ($\beta_2$m-/-) do not express H-2K$^b$ or other non-classical class I molecules, yet they express low levels of H-2D$^b$ heavy chain in the absence of
30 $\beta_2$m. To derive fully H-2 knockout mice, Prof. F. Lemonnier (Pasteur Institute, Paris), prepared H-2D$^{b-/-}$ mice. These mice were crossed with $\beta_2$m$^{-/-}$ mice and bred to derive homozygous $\beta_2$m$^{-/-}$, D$^{b-/-}$ mice that do not express any H-2 class I. These mice are practically depleted of CD8$^+$ splenocytes, as well as other CD8$^+$ cells. To reconstitute in these mice expression of a stable HLA-A2.1, expression
35 of $\beta_2$m is necessary. A construct containing a leader sequence, domains $\alpha$1 and $\alpha$2 of HLA-A2.1 and $\alpha$3, transmembrane and cytoplasmic domains of H-2D$^b$ fused to human $\beta_2$m (HhD) was prepared. The exchange of the $\alpha$3 human domain by a murine domain in HhD is thought to improve the interaction of the class I

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molecule with CD8 molecules of the murine CTL (29). This HhD construct was transfected into RMA and RMA-S cells and shown to bind HLA-A2.1 restricted peptides. The HhD construct was used to produce transgenic mice in C57BL/6 recipients and positive founder mice were bred to the $\beta 2m^{-/-}$, $D^{b-/-}$ mice (30).

The $\beta 2m^{-/-}$, $D^{b-/-}$, $HhD^{-/+}$ heterozygous mice show reconstitution of $CD8^{+}$ cells in the periphery relative to $\beta 2m^{-/-}$ $D^{b-/-}$ mice. Moreover, preliminary data from Prof. Lemonnier's lab showed that CTL induced in HhD mice against influenza NP are directed to the same HLA-A2 dominant epitope as in the human repertoire. Homozygous HhD mice were derived and a colony was established in the Weizmann Institute of Science, Israel.

## SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin (MUC1), and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8 to 10 amino acid residues, of which a second residue from an amino terminal of the peptide and a carboxy terminal residue of the peptide are hydrophobic or hydrophilic natural or non natural amino acid residues, for example, SEQ ID NOs:1-64 and 65 to 77.

According to further features in preferred embodiments of the invention described below, the peptide is derived from Uroplakin, such as Uroplakin II, Uroplakin Ia, Uroplakin III and Uroplakin Ib, for example, SEQ ID NOs:1-19 or 50-64.
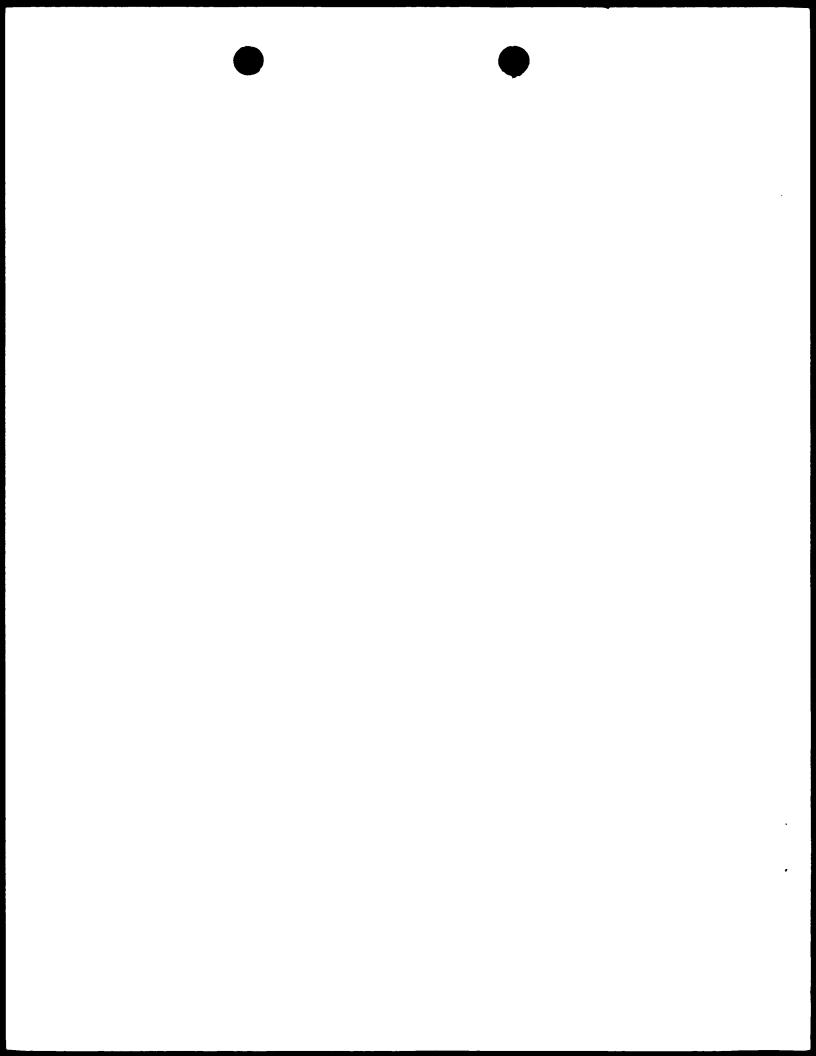
According to still further features in the described preferred embodiments the peptide is derived from the Prostate specific antigen (PSA), for example, SEQ ID NOs:20-24.

According to still further features in the described preferred embodiments the peptide is derived from the Prostate specific membrane antigen (PSMA), for example, SEQ ID NOs:25-30.

According to still further features in the described preferred embodiments the peptide is derived from the Prostate acid phosphatase (PAP), for example, SEQ ID NOs:31-34.

According to still further features in the described preferred embodiments the peptide is derived from the Mucin, for example, SEQ ID NOs:42-49.

According to still further features in the described preferred embodiments the peptide is derived from a non tandem repeat array of the Mucin.

According to still further features in the described preferred embodiments the peptide is derived from a region selected from the group consisting of a signal peptide, a cytoplasmic domain and an extracellular domain of the Mucin.

According to still further features in the described preferred embodiments the peptide is derived from the Lactadherin (BA-46), for example, SEQ ID NOs:35-41.

According to still further features in the described preferred embodiments, the peptide is derived from the Teratocarcinoma-derived growth factor (CRIPTO-1), for example, SEQ ID NOs. 66 to 77.

According to still further features in the described preferred embodiments the Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin (MUC1) and Teratocarcinom-derived growth factor (CRIPTO-1) are each independently of mammalian origin.

According to still further features in the described preferred embodiments the mammal is selected from the group consisting of a humanoid and a rodent.

According to still further features in the described preferred embodiments the peptide includes at least one non-natural modification.
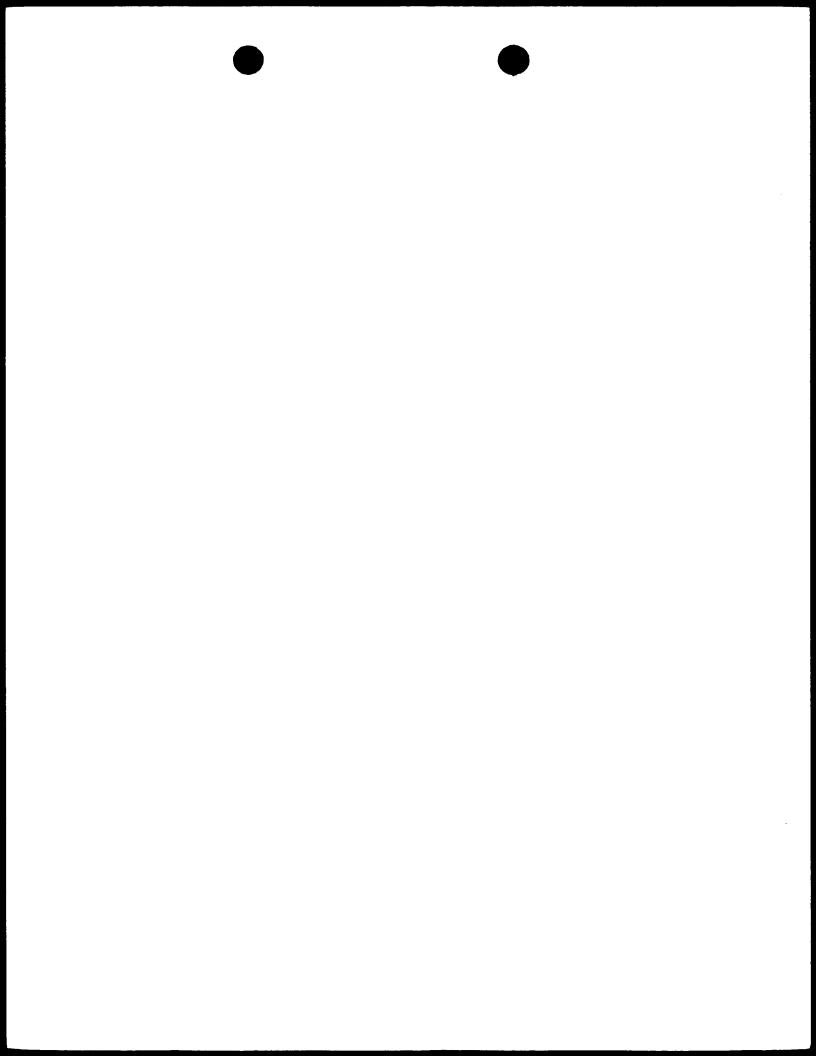
According to still further features in the described preferred embodiments the peptide includes at least one non-natural modification rendering peptides more immunogenic or more stable.

According to still further features in the described preferred embodiments the at least one modification is selected from the group consisting of peptoid modification, semipeptoid modification, cyclic peptide modification, N terminus modification, C terminus modification, peptide bond modification, backbone modification and residue modification.

According to another aspect of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, at least one peptide at set forth herein and a pharmaceutically acceptable carrier.

According to another aspect of the present invention there is provided a vaccine composition comprising, as an active ingredient, at least one peptide at set forth herein and a vaccination acceptable carrier.

According to still further features in the described preferred embodiments the carrier is selected from the group consisting of a proteinaceous carrier to which the at least one tumor associated antigen peptide is linked, an adjuvant, a protein or a recombinant protein and an antigen presenting cell.

According to still further features in the described preferred embodiments the composition, pharmaceutical or vaccine, is effective in prevention or cure of cancer or carcinoma metastases.

According to still further features in the described preferred embodiments the cancer is selected from the group consisting of breast, bladder, prostate, pancreas, ovary, thyroid, colon, stomach and head and neck cancer.

According to still further features in the described preferred embodiments the cancer is a carcinoma.

Further according to the present invention there is provided a method of prevention or cure of a cancer or of metastases thereof comprising the step of administering to a patient an effective amount of the pharmaceutical composition described herein.
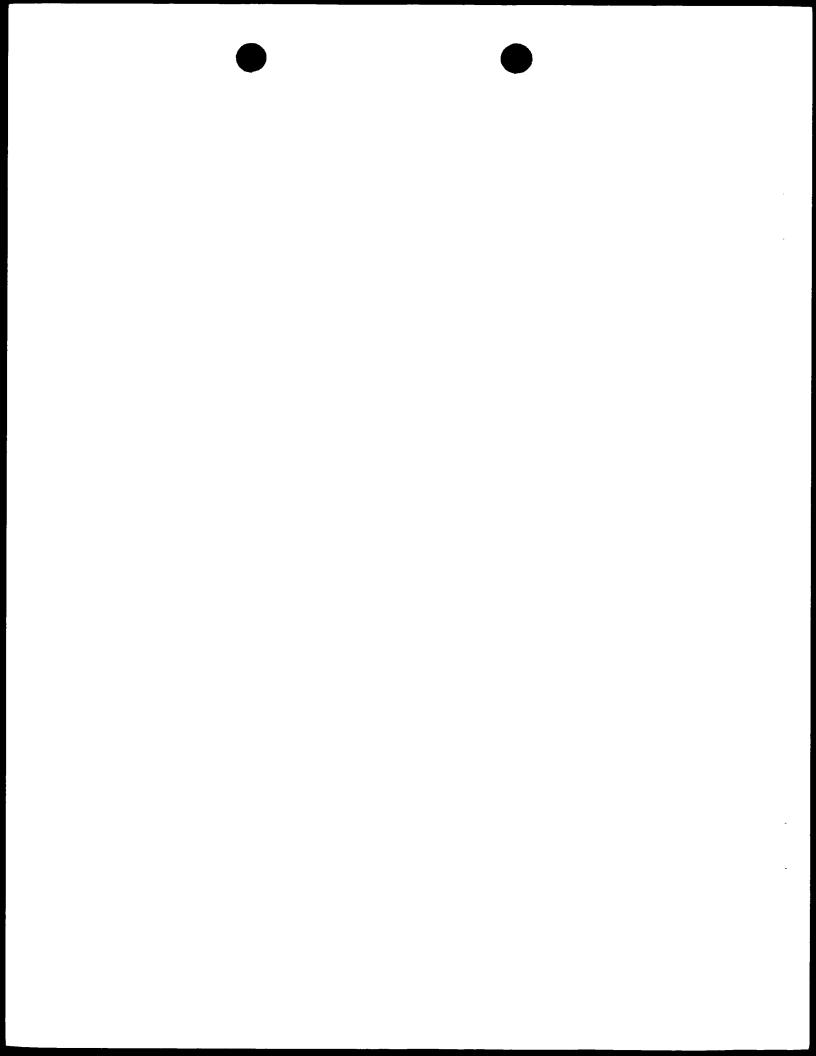
Still further according to the present invention there is provided a method of prevention or cure of a cancer or of metastases thereof comprising the step of vaccinating a patient with an effective amount of the vaccine composition described herein.

According to yet another aspect of the present invention there is provided a polynucleotide encoding at least one peptide as set forth herein. One ordinarily skilled in the art would know how to reverse translate any of the peptides according to the present invention such as the peptides set forth in SEQ ID NOs:1-64 and 66 to 77, using, for example, the human preferred codon usage, to derive the sequences of the polynucleotides according to the present invention. Such polynucleotides are readily synthesisable using the well known solid phase technology for preparation of oligonucleotides such as oligodeoxynucleotides or analogs thereof.

According to still further features in the described preferred embodiments the polynucleotide forms a part of a longer polynucleotide designed to encode a fused protein product from which at least one peptide is cleavable by a protease.

According to a further aspect of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, at least one polynucleotide at set forth herein and a pharmaceutically acceptable carrier.

According to still a further aspect of the present invention there is provided a cellular vaccine composition comprising an antigen presenting cell presenting at least one peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the at least one peptide comprising 9 or 10 amino acid residues, of which a second residue from

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an amino terminal of the peptide and a carboxy terminal residue of the peptide are hydrophilic or aliphatic non-charged natural or synthetic amino acid residues.

According to still further features in the described preferred embodiments the antigen presenting cell is selected from the group consisting of a dendritic cell,
5   a macrophage, a B cell and a fibroblast.

According to still further features in the described preferred embodiments the antigen presenting cell is caused to present the at least one tumor associated antigen peptide by a method selected from the group consisting of (a) genetically modifying the antigen presenting cell with at least one polynucleotide encoding
10   the at least one tumor associated antigen peptide such that the said peptide or at least one longer polypeptide including said peptide will be expressed; (b) loading the antigen presenting cell with at least one polynucleotide encoding the at least one tumor associated antigen peptide; (c) loading the antigen presenting cell with the at least one tumor associated antigen peptide; and (d) loading the antigen
15   presenting cell with at least one longer polypeptide including the at least one tumor associated antigen peptide.

According to a still further aspect of the present invention there is provided a pharmaceutical composition also comprising a helper peptide.

According to a still further aspect of the present invention there is provided
20   a pharmaceutical composition, wherein the helper peptide has a T helper epitope.

According to a still further aspect of the present invention there is provided a vaccine composition also comprising a helper peptide.

According to a still further aspect of the present invention there is provided a vaccine composition, wherein the helper peptide has a T helper epitope.
25   According to a still further aspect of the present invention there is provided a use of the at least one peptide in the manufacture of a medicament.

According to a still further aspect of the present invention there is provided the at least one peptide for use as a medicament.

According to a still further aspect of the present invention there is provided
30   a use of the at least one peptide in the manufacture of a medicament for the prevention or cure of a cancer or cancer metastases.

According to a still further aspect of the present invention there is provided the at least one peptide for use as a medicament for the prevention or cure of a cancer or cancer metastases.
35   According to a still further aspect of the present invention there is provided a peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin

(MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8-10 amino acid residues as selected so as to promote effective binding to a MHC class 1 type molecule such that a CTL response is elicitable.

The present invention successfully addresses the shortcomings of the presently known configurations by providing novel tumor associated antigen peptides effective in eliciting CTL response which may therefore be effective therapeutic agents to combat cancer.

## BRIEF DESCRIPTION OF THE DRAWINGS
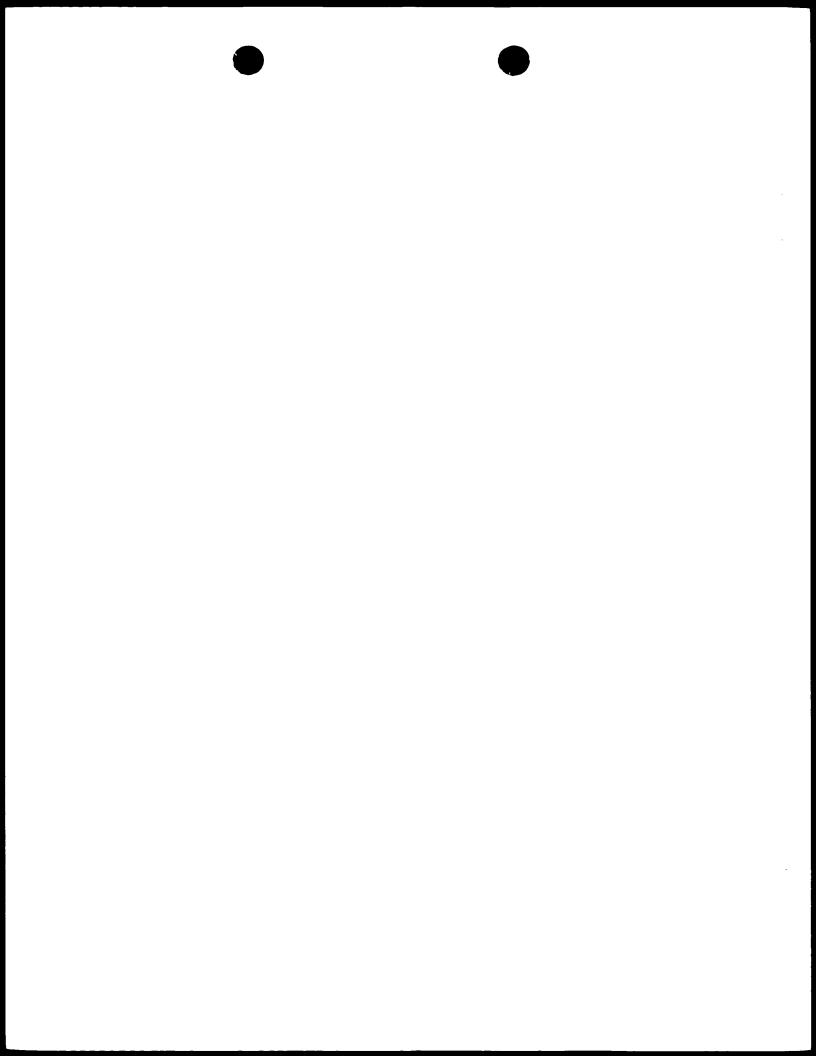
The invention herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1 demonstrates stabilization of cell surface HhD on RMA-S cells by human Uroplakin II derived peptides. Uroplakin II derived synthetic peptides (HURO1-7) or a peptide from the melanoma associated TAA Tyrosinase (Tyr) which served as a control were loaded at various concentrations (3-300 μM) on the RMA-HhD cells as described. Indirect fluorescence activated cell sorter (FACS) analyses were performed by incubating $5 \times 10^5$ loaded cells with the anti-HLA-A2 monoclonal antibodies BB7.2 for 30 minutes at 4° C. After washing the cells in PBS-0.5 %, BSA-0.1 %, sodium azide, the second antibody, goat anti mouse-FITC was applied for 30 minutes at 4 C. Following another wash the fluorescence was recorded on a FACS scan (BD). Mean fluorescence at 300 μM is shown.

FIG. 2 demonstrates lytic activity of human peripheral blood lymphoyctes (PBL) against Uroplakin II loaded target cells. PBL from HLA-A2 positive patients were resensitized with tumor extracted peptide preparations from transitional cell carcinoma (TCC) specimens as described in Materials and Methods. Resensitized lymphocytes from 4 TCC patients treated with Bacillus Calmette Guerein (BCG), 2 non-treated TCC patients, and 1 prostate cancer patient (control), were admixed at different ratios with labeled T2 cells, loaded with TCC extracted peptides (TCC pool) normal bladder mucosa extracted peptides (bladder), synthetic Uroplakin II peptides (HURO1-7) or the melanoma peptide from Tyrosinase (Tyr). Labeled target cells, J82, a HLA-A2 expressing TCC line, SUP, a low class I expressor and K562, a NK sensitive line were also tested. Percent specific lysis, at effector: target of 100:1 is presented.

FIG. 3 demonstrates lytic activity of human peripheral blood lymphocytes against peptides extracted from individual TCC tumors. PBL were resensitized as in Figure 2. Peptides extracted from 9 individual tumors (TCC1-9), that were

pooled for resensitization, were loaded on T2 cells. Labeled loaded targets were tested for lysis by sensitized PBL as described in Figure 2.
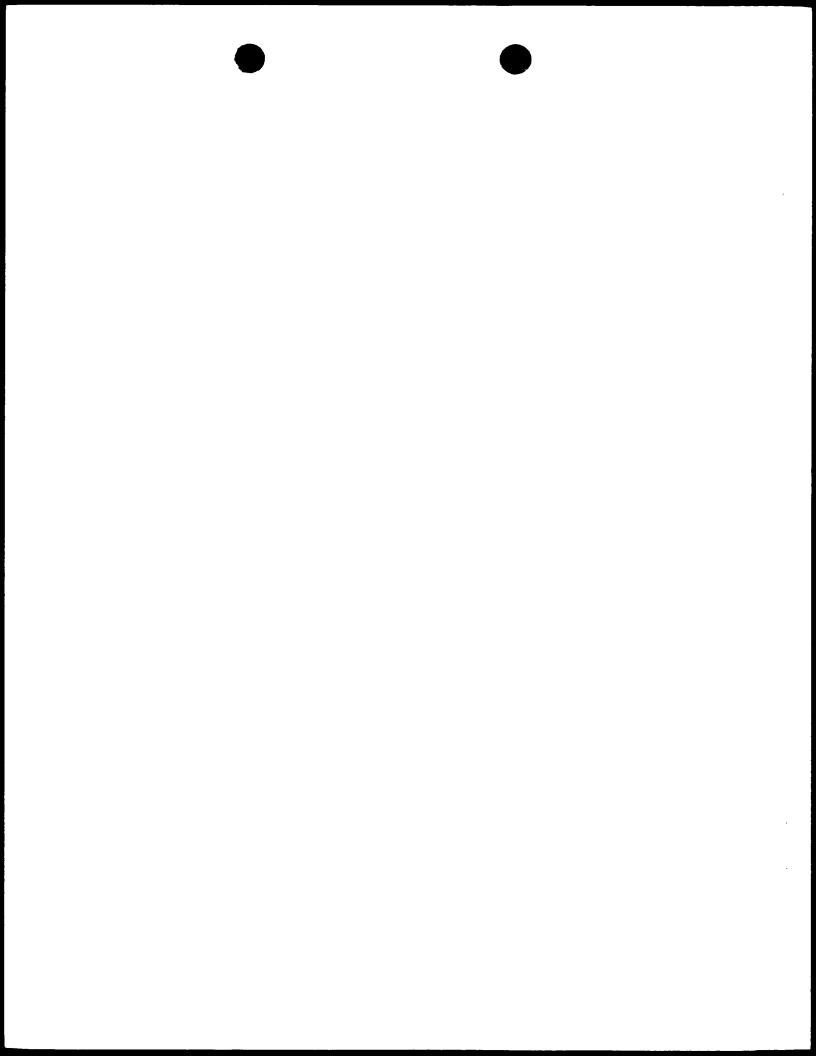
FIG. 4 demonstrates lysis of human Uroplakin II and murine Uroplakin II peptide loaded target cells by CTL induced again human Uroplakin II derived peptides in HhD mice. Mice were immunized as described, by RMA-S-HhD-B7.1 cells, loaded with human Uroplakin II derived synthetic peptides 1-7 as described in Materials and Methods. Loading was performed with individual peptides and cells were pooled for immunization. Resensitized CTL (see Materials and Methods) were tested for lysis of RMA-S-HhD targets loaded with human or murine Uroplakin II peptides (HURO 1-7, MURO1, 3, 4, 6) with TCC or normal bladder extracted peptides (RMA-S TCC, RMA-S bladder), with the tyrosinase peptide (RMA-S Tyr) with peptides derived from breast tumors (RMA-S Br.) or with the cell lines J82 or SUP. E:T ratios of 100:1, 50:1, 25:1 and 12.5:1 were tested and the results are presented in lytic units LU30 was calculated by linear regression analysis of percentage lysis vs. the log (ln) of effector cell number and expressed as the number of LU per $10^6$ effector cells. The coefficient was 0.85 - 0.95 for all groups.

FIG. 5 demonstrates lysis of human Uroplakin II and Ia and murine Uroplakin II derived peptide loaded target cells by CTL induced against TCC extracted peptides in HhD mice. HhD mice were immunized by RMA-S-HhD-B7.1 cells loaded with peptides extracted from 9 TCC samples and pooled. CTL were prepared as described and tested for lysis of labeled RMA-S-HhD target cells loaded with synthetic, tissue derived or control peptides and cell lines as in Figure 4 and synthetic peptides derived from Uroplakin Ia (HURO 11-18). The results are presented in lytic units as described in Figure 4.

FIG. 6 demonstrates stabilization of cell surface HhD on RMA-S cells by human Prostate specific antigen (PSA) and Prostate acid phosphatase (PAP) derived peptides. PSA and PAP derived peptides were loaded at various concentrations (1 μM - 1 mM) on RMA-S-HhD cells. FACS analysis was performed as described in Figure 1, wherein Tyr served as a control. Mean fluorescence at 1 mM is presented.

FIG. 7 demonstrates stabilization of cell surface HhD on RMA-S cells by human Prostate specific membrane antigen (PSMA) derived peptides. PSMA derived peptides (1 μM - 1 mM) were loaded and cells were monitored as described in Figure 6.

FIG. 8 demonstrates immunogenicity and antigenicity of PAP derived peptides in HhD mice. HhD mice were immunized as described, by RMA-S-HhD-B7.1 cells loaded individually with each PAP peptide and pooled

before vaccination. CTL were tested against RMA-HhD cells loaded with synthetic peptides (PAP 1-4), prostate carcinoma extracted peptides (RMA-S, CAP), hyperplastic (normal) prostate extracted peptides (RMA-S HP), breast carcinoma derived peptides (RMA-S Br, control) and tyrosinase (RMA-S Tyr, control). The prostate carcinoma cell line DU145 (DU) and its HhD transfectant (DU HhD) were also tested as targets. The results are presented in lytic units as in Figure 4.

FIG. 9 demonstrates immunogenicity and antigenicity of PSA derived peptides in HhD mice. Procedures were performed and results are presented as in Figure 8.

FIG. 10 demonstrates immunogenicity and antigenicity of PSMA derived peptides in HhD mice. Procedures were performed and results are presented as in Figure 8.
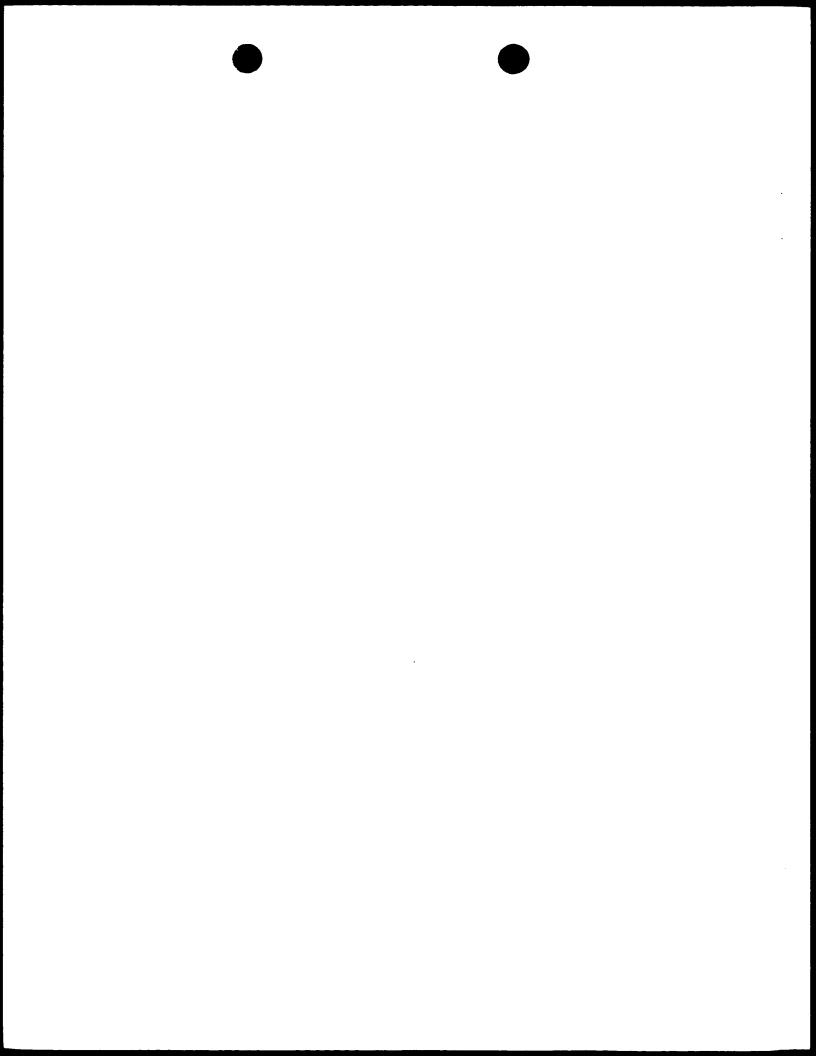
FIG. 11 demonstrates lysis of PSA, PAP and PSMA derived peptide loaded RMA-S-HhD cells by CTL induced against DU145-HhD cells in HhD mice. Mice were immunized with irradiated DU145-HhD cells and CTL assays were performed as described in Materials and Methods. Labeled RMA-S-HhD target cells were loaded with synthetic PSA (1-5), PAP (1-4), PSM-A (1-6) or tyrosinase (RMA-S Tyr) peptides, with prostate carcinoma (RMA-S CAP), breast carcinoma (RMA-S Br.) or hyperplastic prostate (RMA-S HP) extracted peptides. DU145 (DU) and DU145-HhD also served as targets. The results are presented in lytic units as in Figure 4.

FIG. 12 demonstrates lysis of PSA, PAP and PSMA derived peptide loaded RMA-S-HhD by CTL induced against prostate carcinoma extracted peptides in HhD mice. HhD mice were immunized with RMA-S-HhD-B7.1 cells loaded with peptides extracted from prostate carcinoma samples. Experimental details are equivalent to the details in Figure 11.

FIG. 13 demonstrates stabilization of cell surface HhD on RMA-S- cells by human BA-46 (Lactadherin) derived peptides. RMA-S-HhD cells were incubated with 1-100 μM synthetic peptides and monitored as described in Figure 1.

FIG. 14 demonstrates immunogenicity of BA-46 peptides in HhD mice. Mice were immunized as described in Materials and Methods, with RMA-S-HhD-B7.1 cells loaded with synthetic peptides. CTL assays were performed on RMA-S-HhD target cells loaded with homologous peptides.

FIG. 15 demonstrates lysis of BA-46 peptide loaded targets by CTL induced against breast carcinoma extracted peptides in HhD mice. HhD mice were immunized with RMA-S-HhD-B7.1 cells loaded with peptides extracted

from breast carcinoma samples. Experimental details are as in Figure 11. The data is presented as percent specific lysis at E:T of 100:1.

FIG. 16 demonstrates differential lysis of tumor extracted peptide loaded target cells by CTL induced again tumor peptides or BA-46 derived peptides in HhD mice. Mice were immunized by RMA-S-HhD-B7.1 cells loaded with synthetic BA-46 derived peptides or breast carcinoma derived peptides as described in Materials and Methods. CTL were tested against RMA-S-HhD cells loaded with breast carcinoma extracted peptides or normal breast tissue extracted peptides. Percent specific lysis at E:T of 100:1 is presented.

FIG. 17 demonstrates HLA-A2.1 (HhD) restricted lysis of a breast carcinoma cell line by CTL induced against BA-46 derived peptides or tumor extracted peptides. HhD mice were immunized as in Figure 16 and CTL were tested against the HLA negative MDA-MB-157 and their HhD transfectants. The data is presented in percent specific lysis at a E:T of 100:1.

FIG. 18 demonstrates stabilization of cell surface HhD on RMA-S- cells by Mucin (MUC-1/A7, E6, D6) derived peptides. Details are as described in Figure 1.
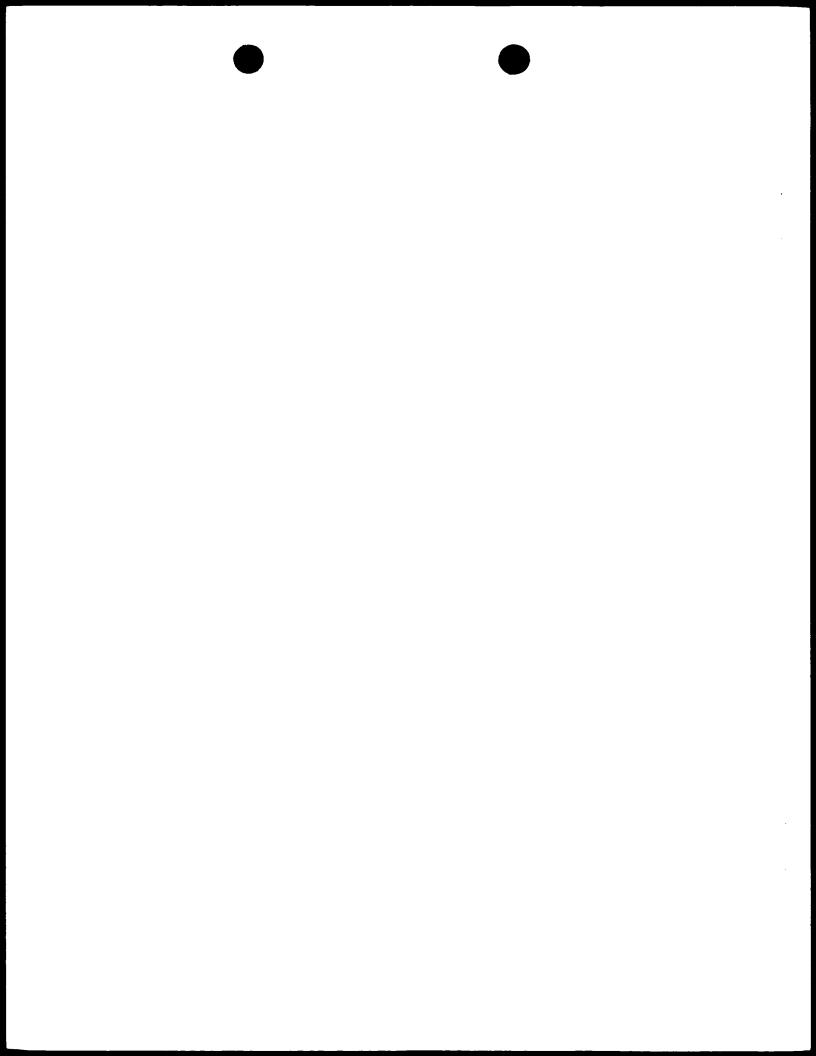
FIG. 19 demonstrates immunogenicity of MUC-1 derived peptides. HhD mice were immunized and CTL tested as in Figure 14.

FIG. 20 demonstrates HLA-A2.1 (HhD) restricted lysis of a breast carcinoma cell line by CTL induced against MUC-1 derived peptides. Details as described in Figure 17.

FIG. 21 demonstrates lysis of MUC-1 loaded target cells by CTL induced against breast carcinoma extracted peptides in HhD mice. Details are as described in Figure 15. E:T is 50:1.

FIG. 22 demonstrates differential lysis of tumor extracted peptide loaded target cells by CTL induced against MUC-1 derived peptides in HhD mice. Details are as described in Figure 16.

FIGs. 23a-e demonstrate lysis of additional human Uroplakin (Ib, II and III) peptide loaded target cells by CTL induced against the peptides in HhD mice. Mice were immunized with RMA-S-HhD-B7.1 cells, loaded with Uroplakin derived synthetic peptides, as described in Materials and Methods. Loading was performed with individual peptides and cells were pooled for immunization. Resensitized CTL (see Materials and Methods) were tested for lysis of RMA-S-HhD targets loaded with Uroplakin peptides or with an irrelevant HLA-A2 binding peptide derived from tyrosinase. Results are expressed as % Lysis using E:T ratios of 100:1, 50:1, 25:1, and 12.5:1. In Figs. 23a-d peptides were loaded onto target cells at a concentration of 200 µM. For Fig. 23e two

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concentrations of peptide were used for loading (20 µM and 200 µM). The protein origins of the peptides examined are listed in Table 3.

FIG. 24a-c demonstrate lysis of human CRIPTO-a peptide loaded target cells by CTL induced against peptides in HhD mice. Mice were immunized with RMA-S-HhD-B7.1 cells, loaded with CRIPTO-1 derived synthetic peptides, as described in Materials and Methods. Loading was performed with individual peptides and cells were pooled for immunization (pool of C1, C3, C5, and C7 for Fig. 24, pool of C2, C4, C6, and C8 for Fig. 24b, and pool of C10-12 for Fig. 24c). Resensitized CTL were tested for lysis of RMA-S-HhD targets loaded with CRIPTO-1 peptides at two concentrations (200 or 20 µM), or with an irrelevant HLA-A2 binding peptide (C2 for Fig. 24a, C10 for Fig. 24b). Results are expressed a % Lysis using E:T ratios of 100:1, 50:1, 25:1, and 12.5:1.
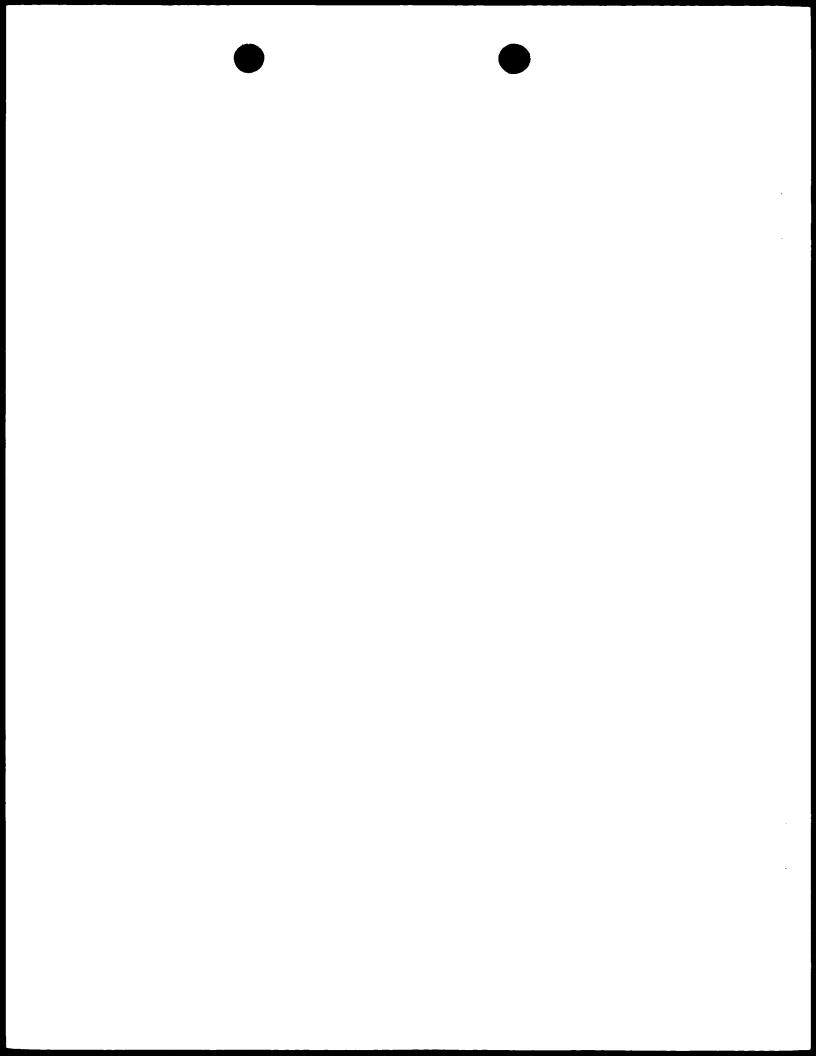
## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention concerns peptides and pharmaceutical and vaccine compositions including same which can be used to prevent or cure cancer, both primary tumors and metastases. Specifically, the present invention can be used to provide vaccines which include novel and potent tumor associated antigen peptides derived from Uroplakins, Prostate specific antigen, Prostate acid phosphatase, Prostate specific membrane antigen, Lactadherin Mucin and Teratocarcinoma-derived growth factor, which can be used as anti-tumor vaccines to prevent or cure, for example, bladder, prostate or breast cancers, carcinomas in particular, or any other tumor expressing the above listed proteins.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

A specific CTL response was found in patients with bladder transitional cell carcinoma (TCC) against the tumor extract and against peptides homologous to Uroplakin II sequence, which was significantly augmented after BCG intravesical therapy. All Uroplakin II peptides employed were good immunizers in HhD mice and CTL from these mice induced intense lysis of targets loaded

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with Uroplakin II homologue peptides, TCC peptide extract and of the J82 HLA-A2 expressing cell line. CTL from HhD mice immunized with TCC peptide extract showed cross reactivity and induced significant lysis of targets loaded with Uroplakin II, or Uroplakin Ia homologue peptides, with TCC peptide extract and

5 of J82 cells. For all immunized HhD mice, there was reduced lysis of targets loaded with normal bladder mucosa peptide extract and of TCC SUP cells (minimal HLA-A2 expression). Also there was no non-specific lysis. In addition, a cross reactivity of CTL induced by human Uroplakin II peptides against murine homologue peptides was found. Despite this finding there was no damage, or

10 inflammatory infiltrate in the internal organs, including the urinary bladder on careful histological examination. These data indicate that Uroplakin homologue peptides constitute specific CTL epitopes and may be considered for immunotherapeutic vaccines alone, or in combination with intravesical BCG instillations in patients prone to recurrence and progression of bladder TCC.

15 Similar results were obtained with human Uroplakin Ib and III.
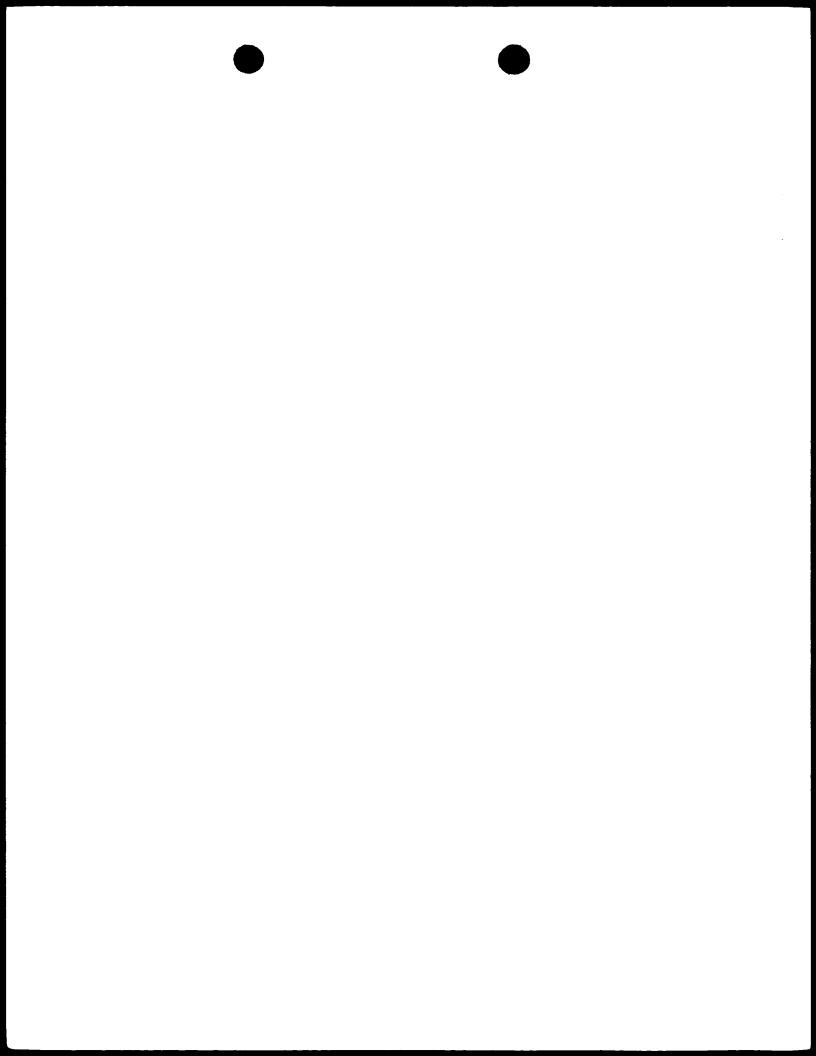
By using HhD mice, HLA-A2 transgenic and which do not express murine MHC class I, it was found that 3 Prostate specific antigen (PSA), 4 Prostate specific membrane antigen (PSMA) and 4 Prostate acid phosphatase (PAP) homologue peptides were immunogenic. The 3 PSA homologue peptides and 2 of

20 the 4 PSMA homologue peptides were not evaluated previously. The 2 PSMA homologue peptides, which were evaluated previously and were found to be CTL epitopes in CAP patients, were also found to be immunogenic in HhD mice. PAP homologue peptides were not evaluated previously and all 4 HLA-A2 binding peptides were very immunogenic in HhD mice and induced also, specific lysis of

25 DU145-HhD cells. Moreover, CTL derived from HhD mice immunized with CAP peptide extract, or with DU145-HhD cells induced specific lysis of targets loaded with CAP peptide extract and PAP homologue peptides. There was also cross reactivity between CTL derived from CAP immunized HhD mice and PSA, or PSMA homologue peptide loaded targets. Low cross reactivity was found for

30 CTL derived from DU145-HhD immunized HhD mice, probably due to low expression of these proteins by DU145 cells. There was no damage, or inflammatory infiltrate in the internal organs of immunized or control HhD mice on careful histological examination. These data indicate that PSA, PSMA and especially PAP homologue peptides constitute specific CTL epitopes and may be

35 considered for immunotherapy of CAP patients.

It was further found a specific CTL response in HhD mice against breast tumor extract and against novel peptides complementary to Lactadherin (BA-46) sequences. These data indicate that BA-46 peptides constitute specific CTL

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epitopes enriched in breast carcinoma and may be considered for immunotherapeutic vaccines.

It was yet further found that Mucin (MUC-1) derived peptides are potential TAA peptides that can be used in anti-tumor vaccine preparations. CTL induced by these peptides lyse better tumor extract loaded targets than normal tissue extract loaded targets. The lysis is HLA-A2 restricted and breast specific. Three novel peptides, from non-tandem repeat array (TRA) domains were shown to constitute potential CTL epitopes.

Thus, in accordance with one aspect of the teachings of the present invention there is provided a vaccine composition which includes at least one tumor associated antigen peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin (MUC1).
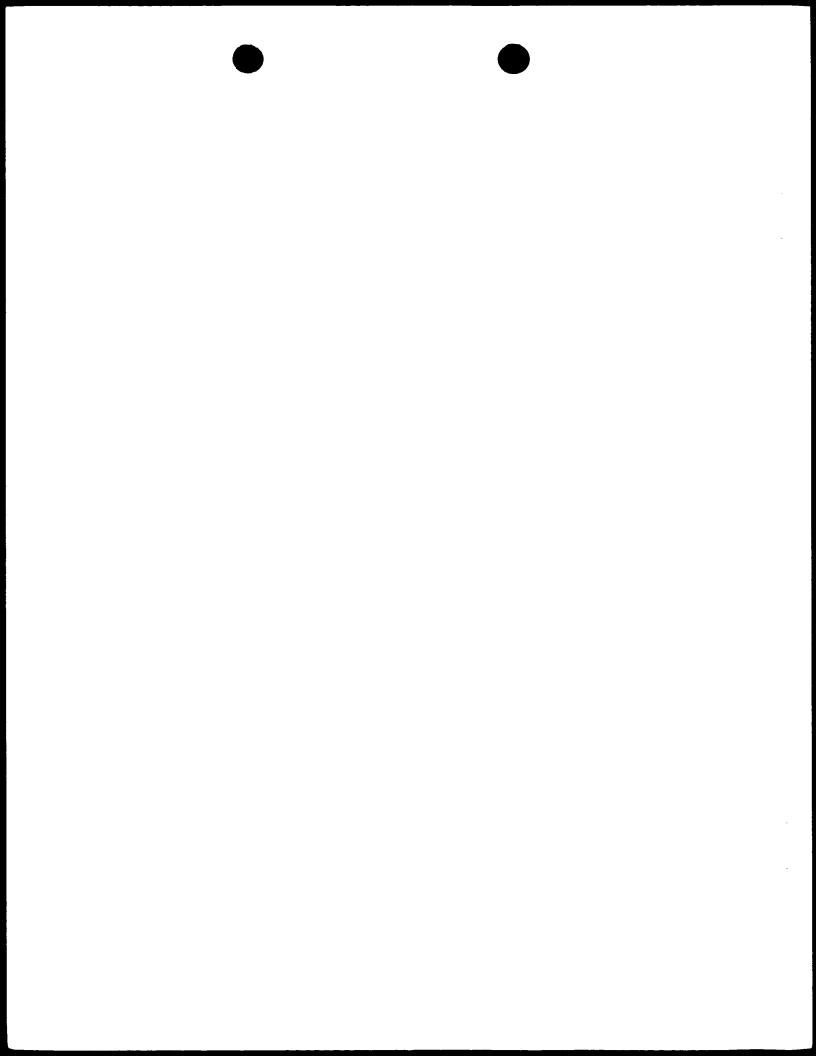
A specific CTL response has been obtained from Teratocarcinoma-derived growth factor (CRIPTO-1) derived peptide in HhD transgenic mice. This data indicates CRIPTO-1 peptides may constitute effective CTL epitopes in humans with HLA-A2 haplotypes and may be considered for immunotherapeutic cancer vaccines.

According to another aspect of the present invention there is provided a method of vaccination for prevention or cure of cancer. The method is effected by administering to a patient a vaccine composition including at least one tumor associated antigen peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin (MUC1).

Immunotherapy by *in vivo* DNA transfer of DNA coding for TAA is based on the rationale of quality or quantity increased peptide presentation leading to activation of an immune response against these peptides. Gene or DNA vaccination results in the intracellular processing and presentation of immunogenic peptides (99). Initial reports on DNA vaccination showed that "naked" DNA injected into the muscle tissue of a mouse is expressed efficiently (32). Embryonically expressed TAA such as CEA was tested (33). Immunization of mice with CEA expressing plasmid DNA was indeed found to protect 100 % of these mice against a challenge with CEA-expressing colon carcinoma cells (34). Both cellular and humoral responses have been reported after DNA vaccination in mice. In other studies a MUC-1 tandem repeat array was used for DNA vaccination of mice and 30% of these mice were protected from a tumor challenge

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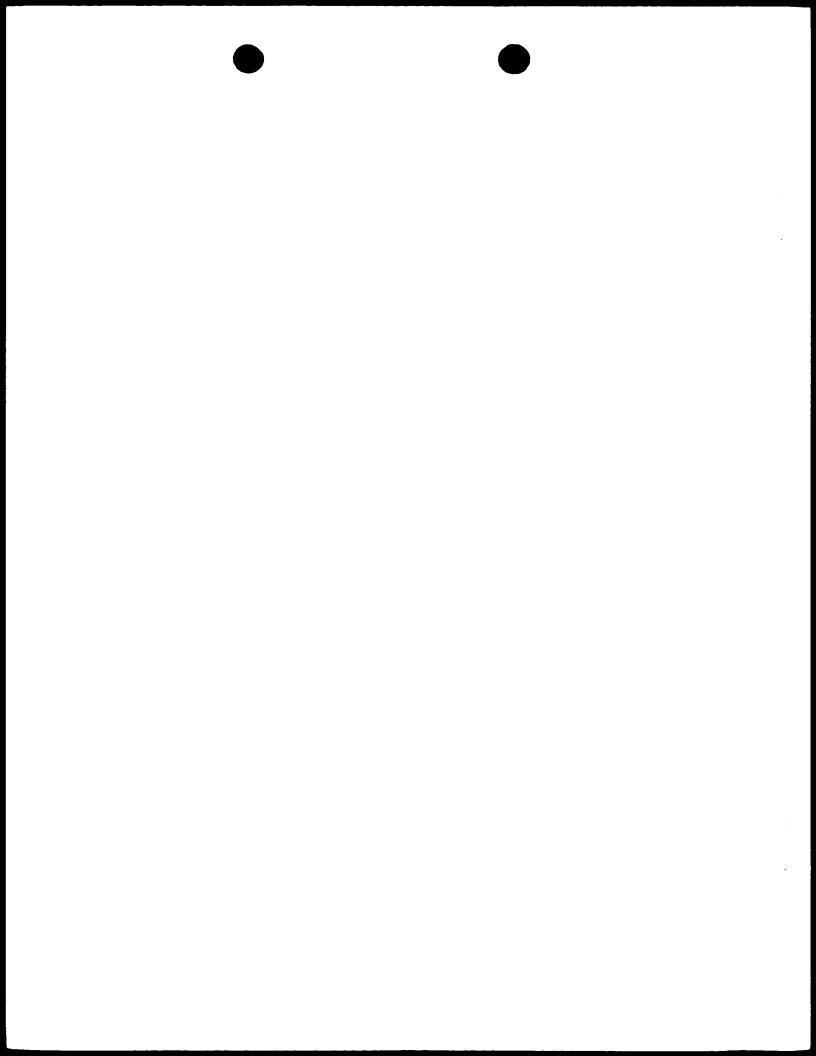
with MUC-1 transfected murine tumor cells (35). DNA vaccination may also be used to elicit immune responses against predefined peptide epitopes, several groups now exploit the string-bead approach to link multiple different CTL or helper epitopes together on the DNA level (36). In some cases the string-bead of 5 peptide coding DNA is built into a vaccinia virus as a delivery vehicle. Recently, it was shown that such a vaccinia virus recombinant poly-epitope vaccine was able to protect mice against several virus infections and a tumor challenge (37). The authors show that all 10 minimal peptide epitopes encoded by the string-bead are expressed and recognized by the appropriate T cell clones (38). RNA was also 10 shown to confer anti-tumor immunity. Vaccination with RNA to ovalbumin induced CTL in mice (39). In conclusion, multiple studies have shown the efficacy of DNA vaccines in anti-viral and anti-tumor immunity.

Thus, according to yet another aspect of the present invention there is provided a DNA vaccine composition which includes at least one polynucleotide 15 encoding a tumor associated antigen peptide derived from a sequence encoding a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46), Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1). As further detailed herein, 20 the at least one polynucleotide can be a part of a longer polynucleotide designed to encode a fused protein product from which the tumor associated antigen peptide is cleavable by a protease.

The polynucleotide is preferably DNA in a form of, or contained in, for example, naked DNA, plasmid, retroviral vector, adenoviral vector, vaccinia viral 25 vector, herpes viral vector, lenti virus vector, EBV vector, CMV vector, polio virus vector, sindbis viral vector, semliki forest virus vector, parvo virus vector, adeno-associated virus vector, virus like particle (VLP) vector. Alternatively, the polynucleotide can be in the form of RNA. The polynucleotide can also be delivered in a non-viral delivery system, such as, for example, but not limited to, 30 in liposomes, in complex with cationic reagents, or with a polycation, such as poly-lysine. The polynucleotide can also be delivered by mechanical means, such as, but not limited to, a gene-gun, by electrical means, or in bacterial vectors like BCG.

There is increasing evidence that peptide vaccination may be much more 35 effective when the peptides are introduced together with an antigen presenting cell (APC) (40). In previous studies of a murine lung carcinoma we have shown that vaccination with a defined TAA peptide (MUT-1) loaded on APC result in long term survival of mice bearing lung metastases (41, 42). The most common cells

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used to load antigens are bone marrow and peripheral blood derived dendritic cells (DC), as these cells express costimulatory molecules that help activation of CTL. Preliminary clinical trials have been performed. In one trial HLA-A1 melanoma patients have been treated with autologous DC loaded with a MAGE-1 peptide.

5 CTL activity was increased in tumor infiltrated lymphocytes (43). In another study, five patients with advanced pancreatic carcinoma were treated with a K-ras derived peptide loaded on DC. As a mutation of K-ras at codon 12 is frequently found in pancreatic carcinoma, three differently mutated peptides, 12-Asp, 12-Arg and 12-Val (non mutated sequence is 12-Gly) were used for vaccination, matched

10 to the mutation in the patient's tumor. Two of the patients showed a specific CTL response and prolonged survival (44). A phase I clinical trial in 51 prostate cancer patients compared a soluble peptide to a DC based peptide in HLA-A2 patients. The peptide was derived from PSMA (SEQ ID NO:29). Only 7 patients that received DC based vaccines with this peptide responded by decreased levels of

15 serum PSA (45). In animal studies a number of groups showed that macrophages loaded with peptides constitute efficient vaccines, yet the number of cells used for vaccination is 10 fold higher than equivalent DC vaccines. Recently in a murine lung carcinoma model the efficacy was tested of syngeneic fibroblasts treated with a proteasome inhibitor to decrease levels of endogenous peptides and loaded with

20 synthetic MUT peptides as vaccines. Effective protection was found against metastatic spread of lung carcinoma.
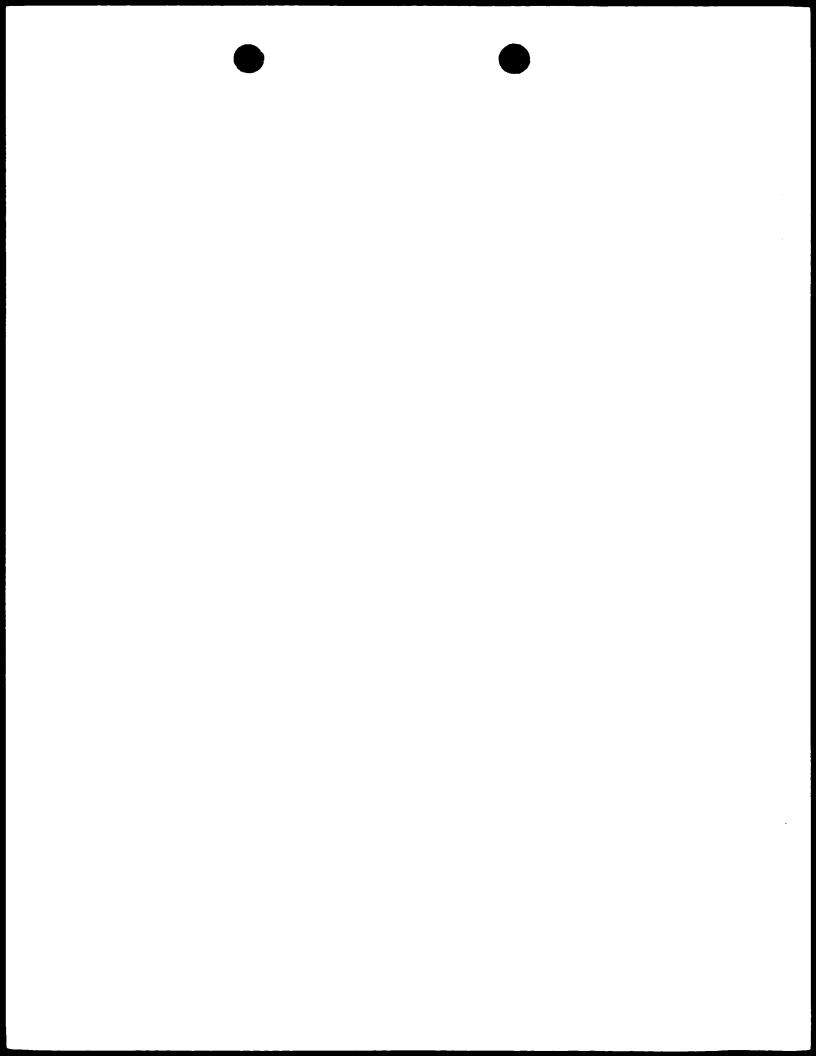
Thus, according to still another aspect of the present invention there is provided a cellular vaccine composition which includes an antigen presenting cell presenting at least one tumor associated antigen peptide derived from a protein

25 selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46), Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1). As further detailed herein, the antigen presenting cell can, for example, be a dendritic cell, a macrophage, a B cell and a fibroblast.

30 Presenting the at least one tumor associated antigen peptide can be effected by a method selected from the group consisting of (a) transducing the antigen presenting cell with at least one polynucleotide (e.g., DNA) encoding the at least one tumor associated antigen peptide; (b) loading the antigen presenting cell with at least one polynucleotide (e.g., RNA) encoding the at least one tumor associated

35 antigen peptide; (c) loading the antigen presenting cell with the at least one tumor associated antigen peptide (e.g., synthetic); and (d) loading the antigen presenting cell with at least one longer polypeptide (e.g., purified) including the at least one tumor associated antigen peptide. Loading can be external or internal. The

polynucleotide, peptide or longer polypeptide can be fused to internalizing sequences, antennapedia sequences or toxoid sequences or to helper sequences, such as, but not limited to, heat shock protein sequences.

5 While it is clear that CD8+ class-I restricted CTL recognize and destroy tumor cells in vitro and in vivo, animal models often show a requirement of CD4+ MHC-class-II restricted T cell help for optimal responses (83). Helper T cell epitopes can contribute to induction of cellular immune responses by class I peptide vaccines, as seen by the synergistic tumor protection upon simultaneous vaccination with T helper and CTL epitopes (84). The 'help' to CTL is most often 10 provided via the production of specific cytokines. Helper epitopes can be specific and derived from a tumor antigen (85). They can also broadly crossreact with a number of MHC class II molecules, and may be either pathogen-derived or comprised of sequences not found in nature (86-88). More specifically, a sequence containing a T helper epitope can be linked to a CTL epitope to create 15 one immunogenic entity. Alternatively, a mixture of 2 or move separate entities, corresponding to CTL and T helper epitopes can be administered to elicit the desired CTL response. T helper epitopes can also be conjugated to other molecules or compounds which increase their biological activity.
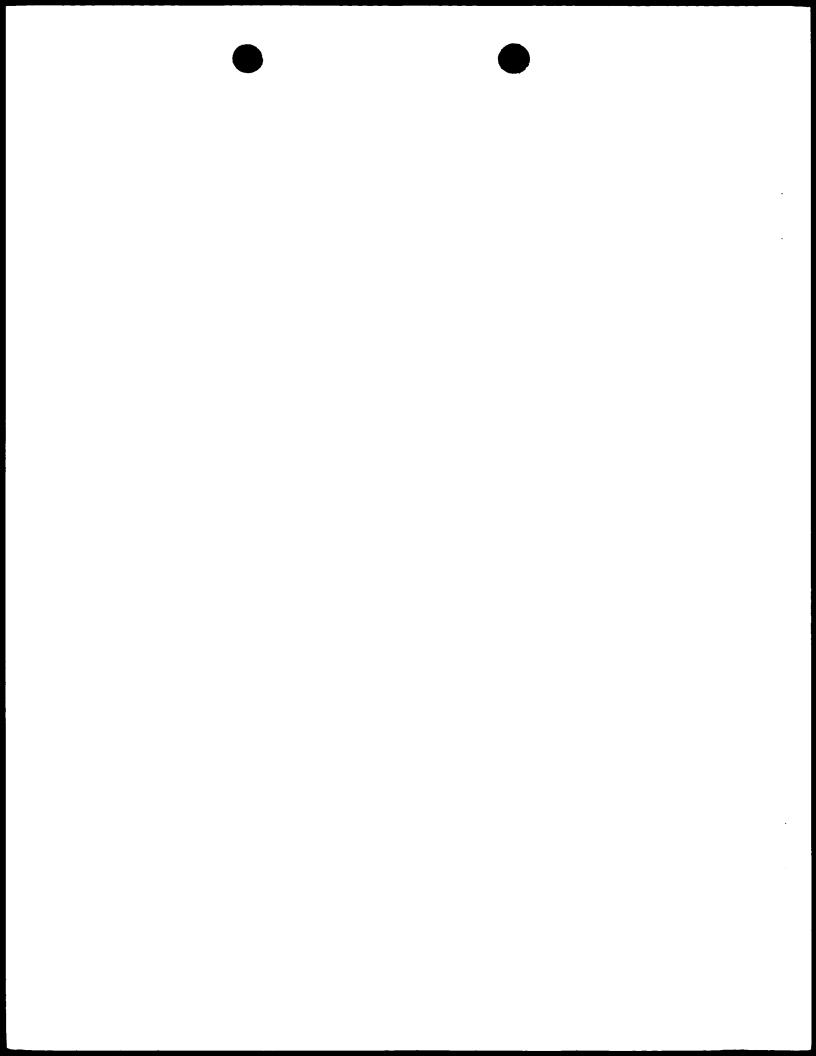
As used herein in the specification and in the claims section below the 20 phrase "tumor associated antigen" also refers to tumor specific antigen.

As used herein in the specification and in the claims section below the term "peptide" refers to native peptides (either degradation products or synthetically synthesized peptides) and further to peptidomimetics, such as peptoids and semipeptoids which are peptide analogs, which may have, for example, 25 modifications rendering the peptides more stable while in a body, or more immunogenic. Such modifications include, but are not limited to, cyclization, N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, $CH_2$-NH, $CH_2$-S, $CH_2$-S=O, O=C-NH, $CH_2$-O, $CH_2$-$CH_2$, S=C-NH, CH=CH or CF=CH, backbone modification and residue 30 modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further detail in this respect is provided herein.

35 As used herein in the specification and in the claims section below the term "amino acid" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including for example hydroxyproline, phosphoserine and phosphothreonine; and other unusual

amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids. Further elaboration of the possible amino acids usable according to the present invention and examples of non-natural amino acids useful in MHC-1 recognizable peptide antigens are given herein.

Peptides can be of 8, 9 or 10 amino acids and peptides of 9 or 10 amino acid residues may be desirable, preferably 9. Thus, assume the following positions (P1-P9) in a 9-mer peptide:

P1-P2-P3-P4-P5-P6-P7-P8-P9

The P2 and P9 positions include the anchor residues which are the main residues participating in binding to MHC class 1 molecules, more specifically HLA-A2. Amino acid resides engaging positions P2 and P9 are hydrophobic or hydrophilic natural amino acids or non-natural amino acids. Examples of natural amino acids being Ala, Cys, Gln, Glu, Ile, Leu, Met, Ser, Thr and Val. These residues may preferably be neutral, hydrophobic, aliphatic and more preferably Val, Leu and Ile. Examples of non-natural amino acids being norleucine (Nle), norvaline (Nva), aminobutyric acid preferably $\alpha$-aminobutyric acid. These residues may preferably be non charged and more preferably aliphatic. P9 can also be an aliphatic amino acid of the general formula $-HN(CH_2)_nCOOH$, wherein n = 2-5, as well as by branched derivatives thereof, such as, but not limited to,
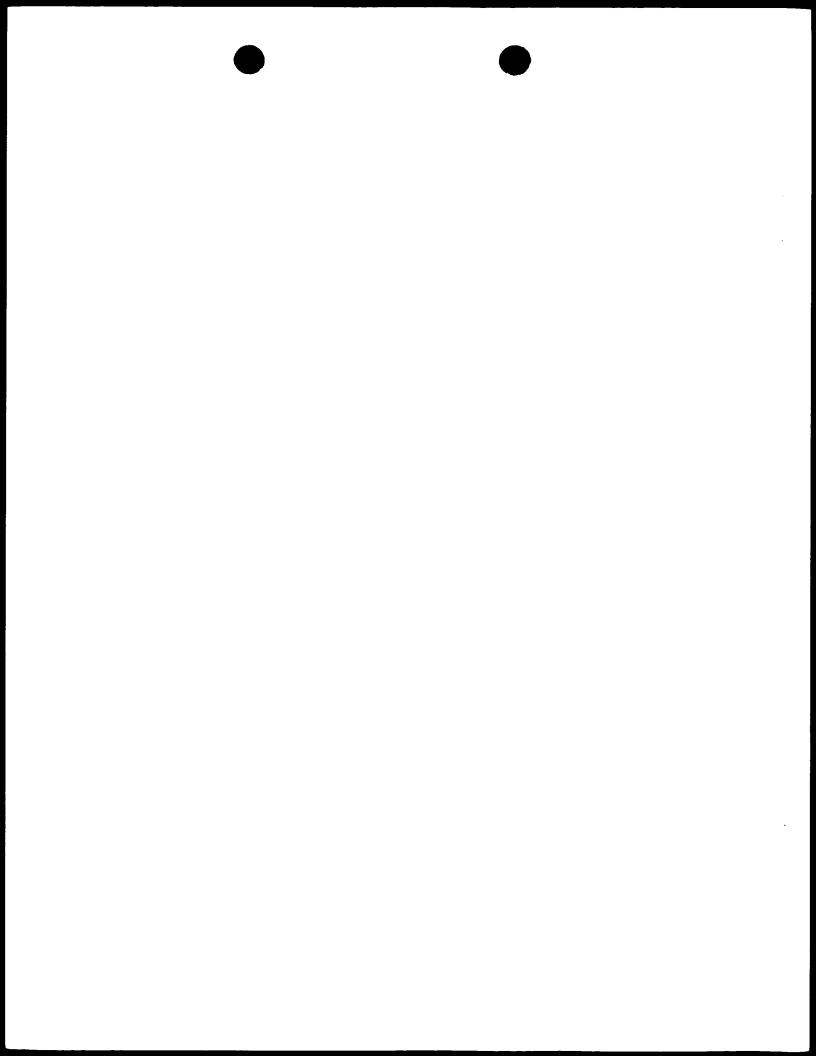
$$-NH(CH_2)_n-COOH$$
$$|$$
$$R$$

wherein R is, for example, methyl, ethyl or propyl, located at any one or more of the n carbons.

Positions P1 and P3 are also known to include amino acid residues which participate or assist in binding to MHC molecules, however, these positions can include any amino acids, natural or non-natural.

The amino terminal residue (position P1) can also be positively charged aliphatic carboxylic acids, such as, but not limited to, $H_2N(CH_2)_nCOOH$, wherein n = 2-5 and $H_2N-C(=NH)-NH(CH_2)_nCOOH$, wherein n = 2-4, hydroxy Lysine, $N^\epsilon$-methyl Lysine, $N^\epsilon$-ethyl Lysine, $N^\epsilon$-propyl Lysine or ornithine (Orn). Additionally, the amino terminal residue can be aromatic residues, such as, but not limited to, phenyl glycine, p-aminophenyl alanine, p-guanidinophenyl alanine or pyridinoalanine (Pal). These latter residues may form hydrogen bonding with the

OH⁻ moieties of the Tyrosine residues at the MHC-1 N-terminal binding pocket, as well as to create, at the same time aromatic-aromatic interactions

5     The other positions P4-P8 are engaged by amino acid residues which typically do not participate in binding to MHC molecules, rather these amino acids are presented to the immune cells. Further details relating to the binding of peptides to MHC molecules can be found in reference 82, see Table V thereof, in particular.

    Amino acid residues engaging position P4-P8 can include any amino acids natural or non natural. These residues may optionally be phosphorylated and/or 10  glycosylated. Indeed residues which have been phosphorylated or glycosylated have been shown in some cases to enhance the binding to the T cell receptor.
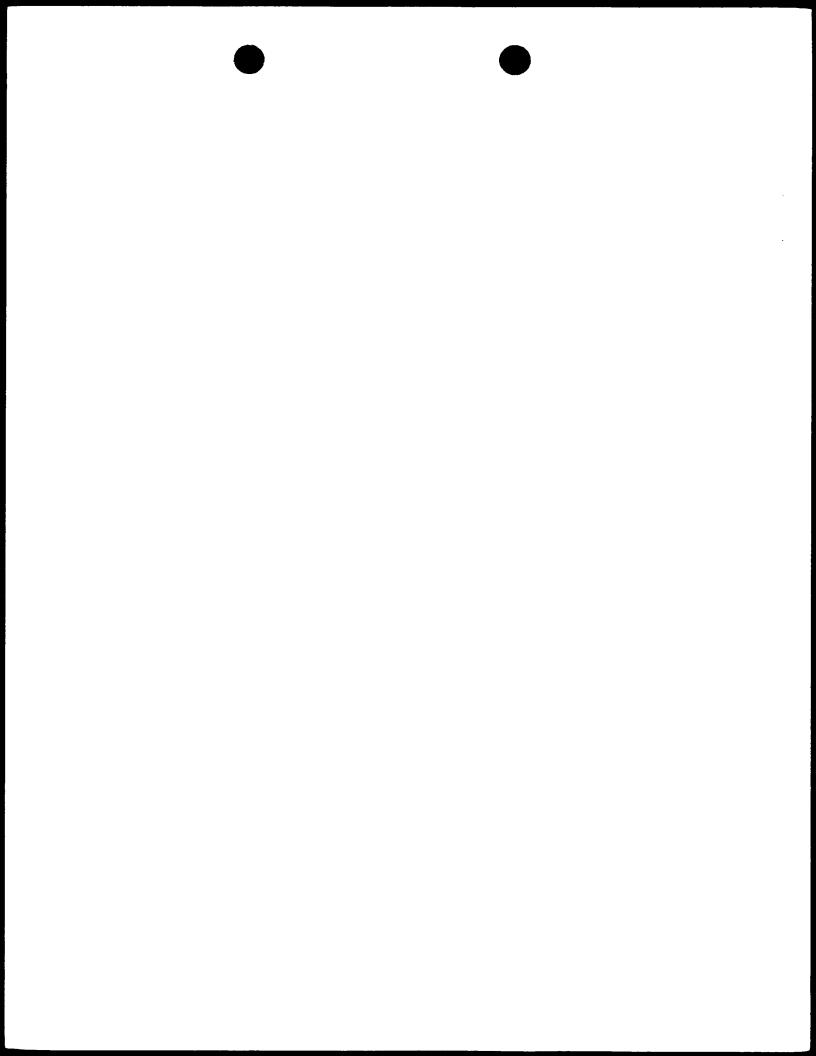
    Cyclization can engage position P4-P8, preferably positions P6 and P7. Cyclization can be obtained through amide bond formation, e.g., by incorporating Glu, Asp, Lys, Orn, di-amino butyric (Dab) acid, di-aminopropionic (Dap) acid at 15  various positions in the chain (-CO-NH or -NH-CO bonds). Backbone to backbone cyclization can also be obtained through incorporation of modified amino acids of the formulas $H-N((CH_2)_n-COOH)-C(R)H-COOH$ or $H-N((CH_2)_n-NH_2)-C(R)H-COOH$, wherein n = 1-4, and further wherein R is any natural or non-natural side chain of an amino acid.

20     Cyclization via formation of S-S bonds through incorporation of two Cys residues is also possible. Additional side-chain to side chain cyclization can be obtained via formation of an interaction bond of the formula $-(-CH_2-)_n-S-CH_2-C(=O)-$, wherein n = 1 or 2, which is possible, for example, through incorporation of Cys or homoCys and reaction of its free SH group with, 25  e.g., bromoacetylated Lys, Orn, Dab or Dap.

    In longer peptides such as in a 10 mer peptide in which the second anchor amino acid is at position P10 the amino acid engaging P9 may include most L amino acids. In some cases shorter peptides such as an 8 mer peptide are also applicable, in which the carboxy terminal acid P8 may serve as the second anchor 30  residue. All the options described for the anchor amino acid residues engaging positions P2 and P9 in a 9 mer peptide may apply likewise to the anchor amino acid residues engaging positions P2 and P10 in a 10 mer peptide and P2 and P8 in an 8 mer peptide.

    The amino acids may be modified as is necessary to provide certain 35  characteristics such as greater immunogenicity, more stability or improved pharmacological properties. The peptides can be for instance subject to changes such as the replacement of one or more amino acid residues whether dissimilar or similar.

20

Modification of the peptides may also be by decreasing, e.g. in a 10 mer peptide, or extending, e.g. in an 8 mer peptide, the amino acid sequence, for example, by deletion or addition of amino acids. It will be appreciated that preferably anchor amino acids should not be deleted.

5      Peptide bonds (-CO-NH-) within the peptide may be replaced by N-alkylated bonds such as N-methylated (-N(CH$_3$)-CO-), ester bonds (-C(R)H-C-O-O-CH(R)-N-), ketomethylen bonds (-CO-CH$_2$-), -aza bonds (-NH-N(R)-CO-), wherein R is hydrogen or any alkyl, e.g., methyl carba bonds (-CH$_2$-NH-), hydroxyethylene bonds (-CH(OH)-CH$_2$-), thioamide bonds
10    (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), and peptide derivatives (-N(R)-CH$_2$-CO-), naturally presented on the carbon atom.
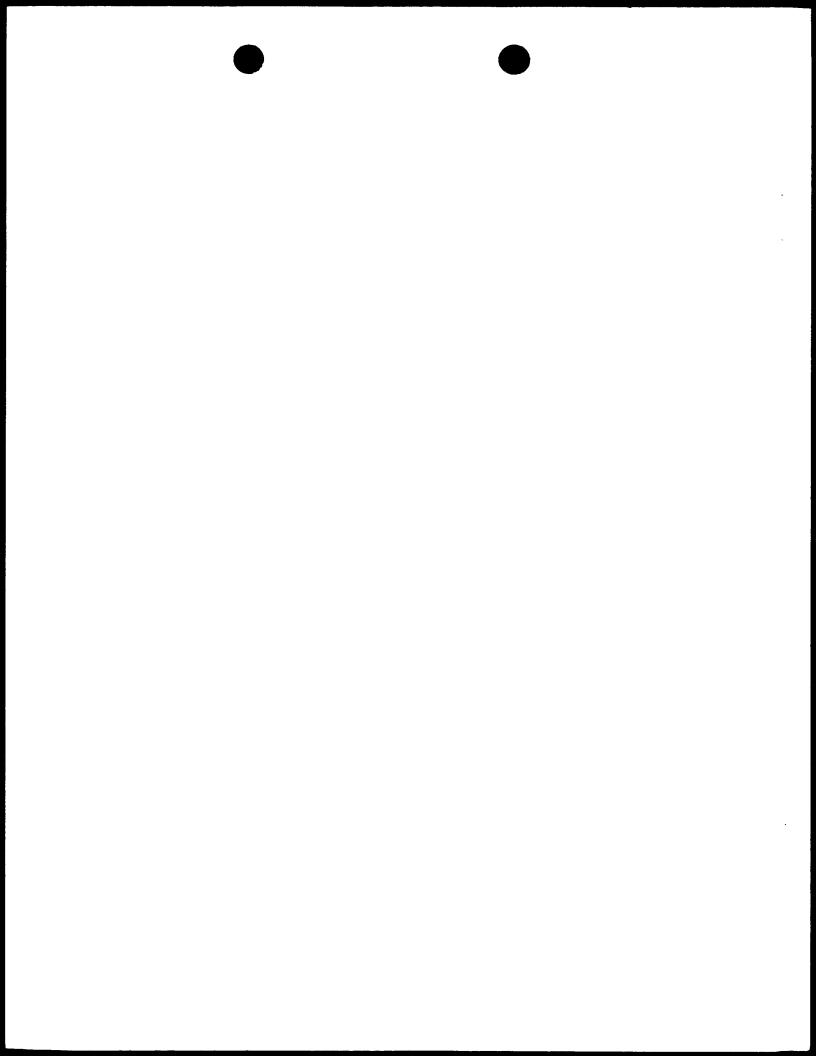
These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time. Preferably, but not in all cases necessary, these modifications should exclude anchor amino acids.

15    For amino acid residues engaging positions other than the second residue from the amino terminal and the end residue at the carboxy terminal natural aromatic amino acids, Trp, Tyr and Phe, may be replaced by synthetic non-natural acid such as TIC, naphthylalanine (Nal), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

20    As used herein in the specification and in the claims section below, the term "transduced" refers to the result of a process of inserting nucleic acids into cells. The insertion may, for example, be effected by transformation, viral infection, injection, transfection, gene bombardment, electroporation or any other means effective in introducing nucleic acids into cells. Following transduction the
25    nucleic acid is either integrated in all or part, to the cell's genome (DNA), or remains external to the cell's genome, thereby providing stably transduced or transiently transduced cells.

As used herein in the specification and in the claims section below the phrase "derived from a protein" refers to peptides derived from the specified
30    protein or proteins and further to homologous peptides derived from equivalent regions of proteins homologous to the specified proteins of the same or other species, provided that these peptides are effective as anti-tumor vaccines. The term further relates to permissible amino acid alterations and peptidomimetics designed based on the amino acid sequence of the specified proteins or their
35    homologous proteins.

As used herein in the specification and in the claims section below the phrase "anti-tumor vaccines" refers to vaccines effective in preventing the development of, or curing, cancer, including primary tumor and/or metastases.

As used herein in the specification and in the claims section below the phrase "prevention or cure" also refers to inhibiting, slowing or reversing the progression of a disease, substantially ameliorating clinical symptoms of a disease or substantially preventing the appearance of clinical symptoms of a disease.

In the specification and in the claims section below the phrase as used herein 'loading' refers to exposing, adding or introducing a substance into or onto a cell or vesicle or part thereof.

According to a preferred embodiment of the present invention the Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46), Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1) proteins are each independently of a mammal, e.g., a humanoid such as a human being or a rodent such as murine.

According to yet another preferred embodiment of the present invention the composition further comprising a carrier. Usually the tumor associated antigen peptide(s) are presented in context of the carrier.

The carrier can be a proteinaceous carrier to which the peptides are linked. Methods of linking short peptides to carriers are well known in the art of vaccination. The carrier can alternatively be a particulate adjuvant, an oil or emulsifier based adjuvant, a gel based type adjuvant, or an adjuvant based on specific targeting of antigen, such as, but not limited to, antibody-liposom conjugates. The carrier can also be a protein or a recombinant protein produced, for example in bacteria, yeast or in mammalian cells, including cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon alpha, interferon beta, interferon gamma and others. The carrier can also be an antigen presenting cell, such as, but not limited to, a dendritic cell, a macrophage, a B cell or a fibroblast. The cell selected is either an autologous or non-autologous HLA matching cell. Optionally the cell can be a cultured cell, a cell treated by various reagents (e.g., by early and/or late acting cytokines), transduced by genes, and/or irradiated or radiated.

The vaccine composition according to the present invention is effective in prevention or cure of cancer and/or cancer metastases. In other words, the composition is effective for primary tumors, secondary tumors and metastases thereof in the same organ or in another organ, provided that the tumor expresses the above listed tumor associated proteins. According to a preferred embodiment of the present invention the cancer being treated or prevented via the administration of the vaccine composition is a carcinoma. i.e., a malignant tumor composed of epithelial tissue. The cancer treated or prevented according to the

present invention can be, for example, breast, bladder, prostate, pancreas, ovary, thyroid, melanoma, colon, stomach and/or head and neck cancer.

According to an embodiment of the present invention, the Uroplakin protein can be Uroplakin II, Uroplakin Ia, Uroplakin III and Uroplakin Ib.

5　　　According to another embodiment of the present invention, the tumor associated antigen peptides derived from the Mucin protein are from a non-tandem repeat array of Mucin.

According to yet another embodiment of the present invention, one or more tumor associated antigen peptides derived from the Mucin protein are from a 10　region selected from the group consisting of a signal peptide, a cytoplasmic domain and an extracellular domain of Mucin.

According to a presently preferred embodiment of the present invention the one or more tumor associated antigen peptides are selected from SEQ ID NOs: 1 to 64 and 66 to 77 and effective homologues and analogs thereof.
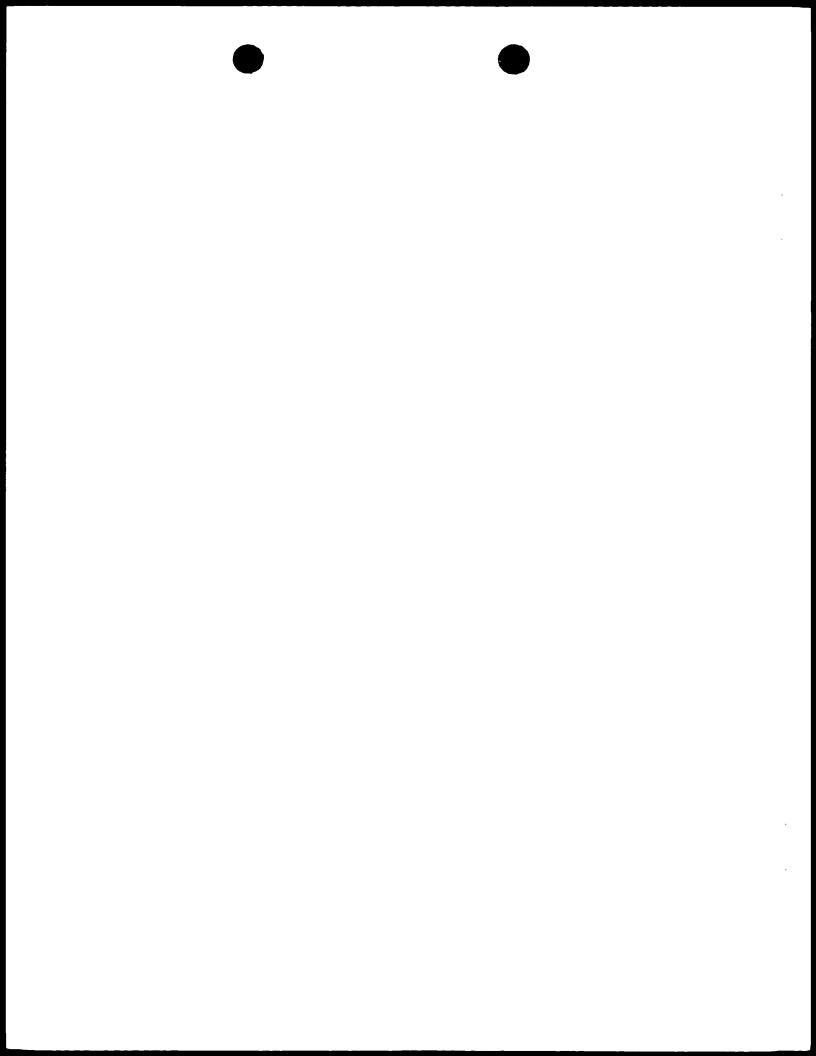
15　　　For therapeutic or prophylactic anti-tumor treatment, the vaccine composition according to the present invention may include thickeners, carriers, buffers, diluents, surface active agents, preservatives, and the like, all as well known in the art.　The composition may also include one or more active ingredients, such as, but not limited to, anti-inflammatory agents, anti-microbial 20　agents, anesthetics and the like.

The vaccine composition may be administered in either one or more ways. Administration may be effected topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip or intraperitoneal, intravesical, subcutaneous, or intramuscular 25　injection.

Compositions for topical administration can include, but are not limited to, lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

30　　　Formulations for parenteral administration can include, but are not limited to, sterile aqueous solutions which may also contain buffers, diluents, adjuvant and other suitable additives.　The adjuvant is preferably of a type allowed for use in treating human beings, such as BCG adjuvant.

Dosing is dependent on responsiveness, but will normally be one or more 35　doses per week or month, with course of treatment lasting from several weeks to several months.　Persons ordinarily skilled in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

The present invention provides novel tumor associated antigen peptides effective in eliciting CTL response which can therefore be effective therapeutic agents to combat cancer.

According to another embodiment of the present invention there is provided a peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8-10 amino acid residues as selected so as to promote effective binding to a MHC class 1 type molecule such that a CTL response is elicitable

Each of the various aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the Examples section that follows.

## EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.
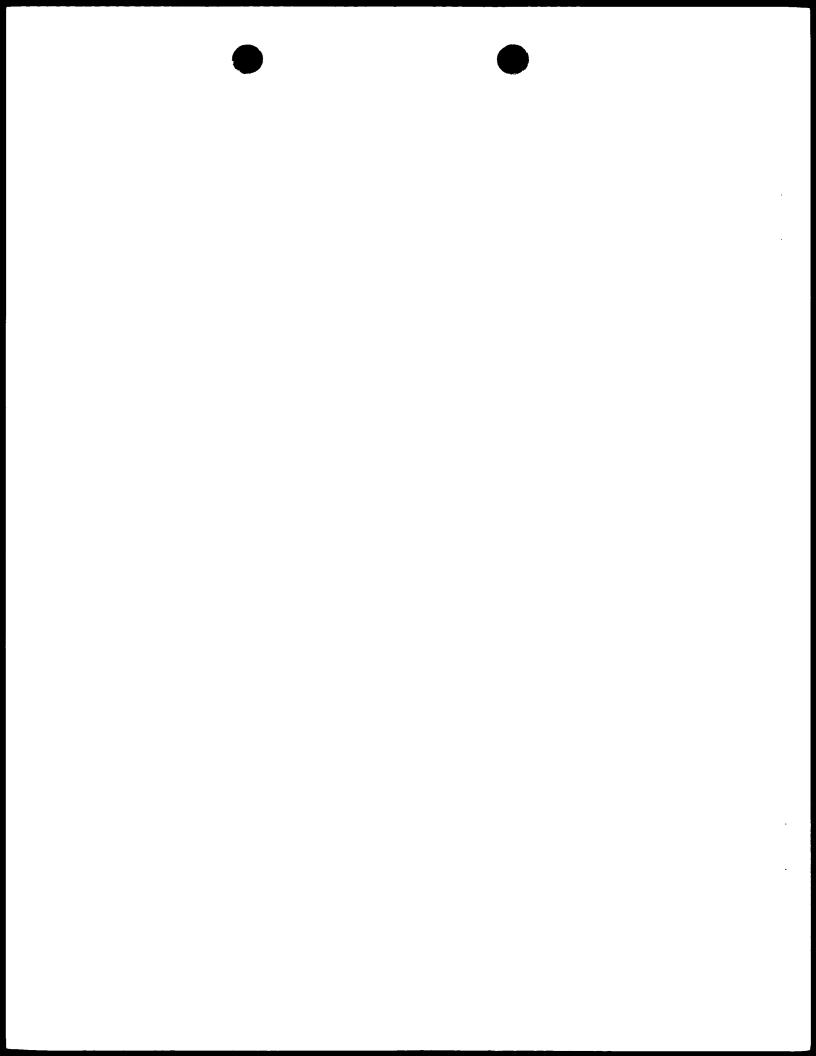
## MATERIALS AND EXPERIMENTAL METHODS

**Mice:** The derivation of HLA-A2.1/$D^b$-$\beta 2$m monochain, transgenics, H-2$D^b$ x $\beta 2$m double knockout mice (HhD mice) was descried before (30).

**Tumor and cell lines:** RMA-S is a TAP-2 deficient lymphoma clone of C57BL/6 origin. The RMA-S HhD and RMA-S-HhD-B7.1 are transfectants carrying the HhD construct without or with the gene for murine B7-1 costimulatory molecule, respectively. T2 is a TAP-2 deficient human lymphoblastoid line expressing HLA-A2. RMA-S, RMA-S derivatives and T2 cells were maintained in RPMI 1640-10 % FCS and antibiotics.

The human tumor cell lines, J82 (TCC of the bladder) sup (TCC of the bladder, DU145 (prostate carcinoma) and MDA-MB-157 (breast carcinoma) and the HhD transfectants of DU145 and MDA-MB-157 were maintained in DMEM-10 % FCS 2 mM glutamine - 1 mM sodium pyruvate - 1 % non-essential amino acids and antibiotics.

HhD and B7-1 transfectants were grown in presence of 500-1000 μg/ml of Geniticin, G418. All tissue culture media and supplements were purchased from Gibco.

**Peptide synthesis:** Peptides were synthesized on an ABIMED AMS 422 multiple peptide synthesizer (Langenfeld, Germany), employing the a-N-9-fluorenylmethoxycartonyl (Fmoc) strategy following the company's commercially available protocols. Peptide chain assembly was conducted on a

5 2-chlorotrityl chloride resin (Novabiochem, Laufelfingen, Switzerland). Crude peptides were purified to homogeneity by reversed-phase HPLC on a semi-preparative silica C-8 column (250 x 10 mm; Lichnonorb RP-8; Merck). Elution was accomplished by a linear gradient established between 0.1 % trifluoroacetic acid in water and 0.1 % trifluoroacetic acid in 70 % acetonitrile in

10 water (v/v). Purity of peptides ( 96 %) was ascertained by analytical HPLC (RP-18) using various acetonitrile water (containing 0.1 % trifluoroacetic acid) gradients. The products' compositions were determined by amino acid analysis (Dionex automatic amino acid analyzer, Sunnyvale, CA) after extraction acid hydrolysis. Molecular weight was ascertained by mass spectrometry (VG

15 Tofspec; Laser Desorption Mass. Spectrometry; Fison Instruments, Manchester, U.K.). Tables 1-8 present these peptides.
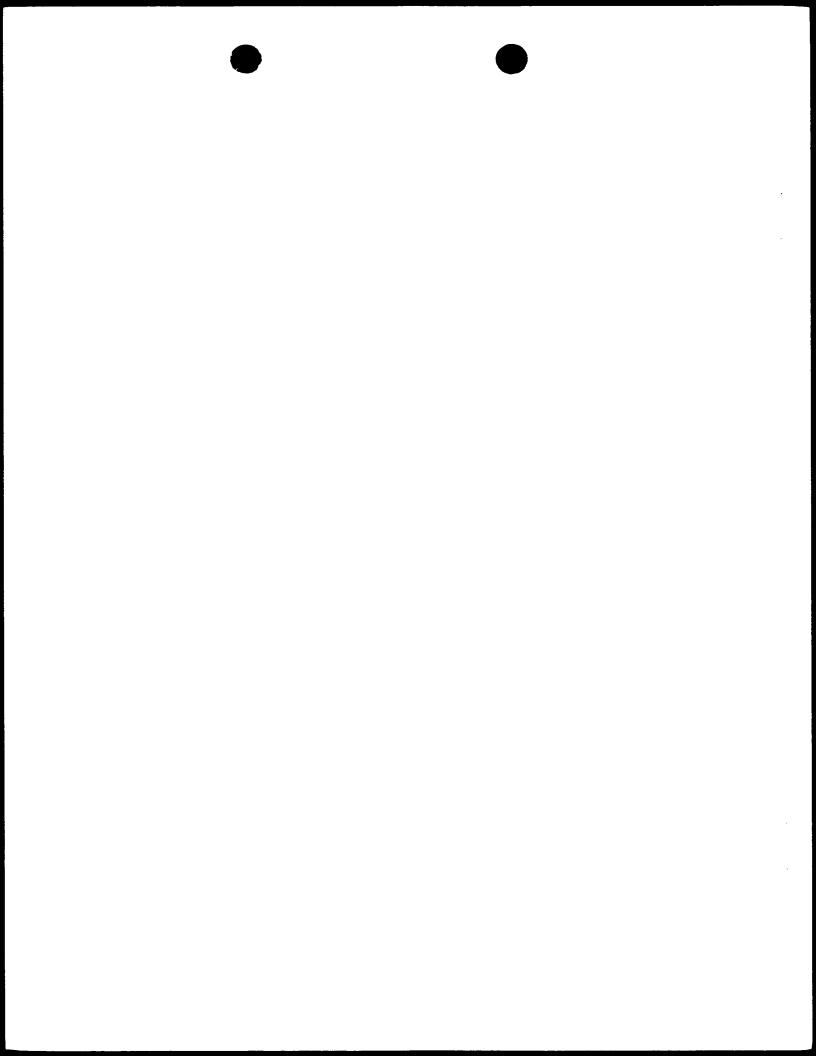
**Preparation of peptide fractions from fresh human tumors and normal tissues:** Surgical specimens of TCC, normal bladder mucosa, prostate carcinoma, hyperplastic benign prostate, breast carcinoma or normal breast tissue were

20 transferred on ice from surgery to a qualified pathologist. Tumor tissue were cleaned from most adjacent tissue and snap frozen. Non-necrotic fragments, from 5-10 patients were homogenized in PBS; 0.5 % Nonidet P-40; 10 μg/ml soybean trypsin inhibitor; 5 μg/ml lupeptide; 8 μg/ml aprotinin and 0.5 mM PMSF and homogenized using a glass-teflon homogenizer. Following further stirring for 30

25 minutes at 4°C, the homogenates were titrated with 10 % TFA to a final concentration of 0.1 % TFA and stirred for 30 minutes at 4°C. After ultra-centrifugation for 30 minutes at 42K rpm the supernatants were applied to Sephadex G25 columns and fractions were monitors at 230 nm. Peptide fractions under 10 Kd were pooled, lyophilized and further fractionated by centriprep 3 by

30 centrifugation (Amicon, Beverly, MA). The peptide pool after lyophilization, was dissolved in opti-MEM (Gibco) for further use.

**Scoring of HLA-A2.1 binding peptides:** Protein sequences were screened for MHC binding by a HLA Peptide Binding Predictions software approachable through a worldwide web interface at (see also reference 82). This software,

35 based on accumulated data, scores every possible peptide in the protein for possible binding to MHC according to the contribution of every amino acid in the peptide. Theoretical binding scores represent calculated half-life of the HLA-A2.1-peptide complex.
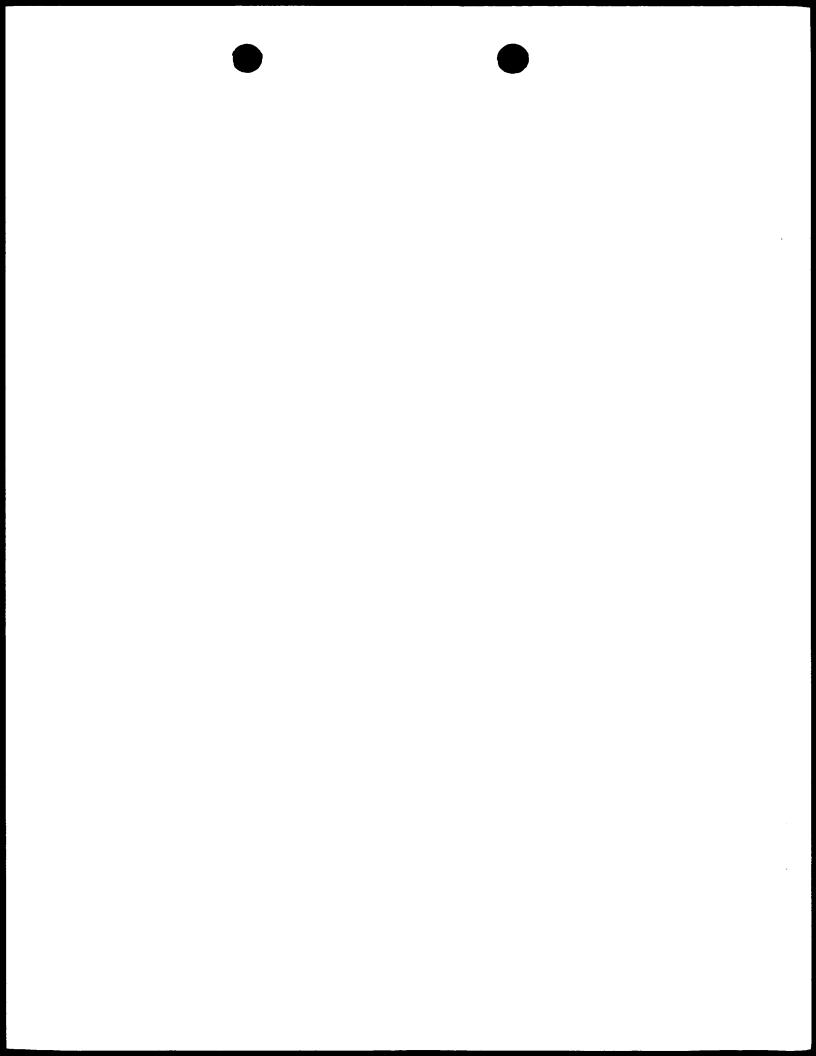
**Measurement of peptide binding by stabilization of cell surface MHC:**
Peptide binding to HhD was measured by stabilization of HhD on RMA-S
transfectants, using an indirect FACS assay as follows: $5 \times 10^5$ peptide loaded
TAP-2 deficient RMA-S-HhD cells (see vaccination), were incubated with anti
HLA monoclonal antibodies for 30 minutes at 4°C. After washing the cells with
PBS - 0.5 %, BSA + 0.1 %, sodium azide, the second antibody, goat anti
mouse-FITC (Jackson Lab.), was applied for 30 minutes at 4°C. Following
washing, the amount of bound antibodies was detected by FACSscan (Bacson).

Mouse monoclonal antibodies B-9-12, w6/32 (anti HLA A, B, C) and
B.B7.2 (anti HLA-A2.1) were used for analyses.

**Vaccination:** Mice were immunized intraperitonealy three times at 7-day
intervals, with $2 \times 10^6$ irradiated (5,000 rad) tumor cells, or with peptide-loaded
RMA-S-HhD-B7.1 transfectants. Peptide loading of RMA-S-HhD-B7.1 cells was
performed as follows: After washing the cells 3 times in PBS, the surface
expression of HhD monochins was stabilized by a 4 hours culture at 26°C.
Synthetic peptides, or peptide extracts were added to $10 \times 10^6$ cells in 1 ml of
opti-MEM (Gibco) medium to a concentration of 100 µM or 1 mM, respectively.
The cells were incubated at 26°C and for additional 3 hours at 37°C. Peptide
loaded RMA-S-HhDσ - σB7.1 cells were irradiated (5000 rad), washed,
resuspended in PBS and injected into mice. In mixed synthetic vaccines,
RMA-S-HhD-B7.1 cells were loaded separately with each peptide and pooled
before vaccination.

***In vitro* cytotoxicity assays:** Mice were immunized intraperitonealy three
times at 7-day intervals, with $2 \times 10^6$ irradiated (5,000 rad) tumor cells, or with
peptide-loaded RMA-S-HhD-B7.1 transfectants. Spleens were removed on day
10 after the last immunization and splenocytes were restimulated *in vitro* with
either irradiated tumor cells (for mice immunized with tumor cells) or with
lymphocytes pulsed with 100 µM synthetic peptides or 1 mM patients-derived
extract in opti-MEM (Gibco) medium. Restimulated lymphocytes were
maintained in RPMI 1640 medium containing 10 % FCS, 1 mM glutamine,
combined antibiotics, 1 mM sodium pyruvate, 10 mM HEPES, pH 7.4, $5 \times 10^{-5}$ M
β-mercaptoethanol, and 1 % nonessential amino acids (RPMI-HEPES medium)
for 5 days. Viable lymphocytes (effector cells), were separated by lymphocyte-M
(Cedarlane, Ontario, Canada) centrifugation, resuspended in RPMI-HEPES
medium, and admixed at different ratios with $5 \times 10^3$ peptide loaded RMA-S-HhD
cells, previously loaded with different peptides (see vaccination). CTL assays
were performed in U-shaped microtiter wells, at 37°C, 5 % $CO_2$ for 5 hours.
Cultures were terminated by centrifugation at 1,000 rpm for 10 minutes at 4°C. A

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total of 100 µl of the supernatants was mixed with scintillation fluids and counted in a beta counter. Percentage of specific lysis was calculated as follows: % lysis = (cpm in experimental well - cpm spontaneous release)/(cpm maximal release - cpm spontaneous release) x 100. Spontaneous release was determined by incubation of 100 µl-labeled target cells with 100 µl of medium. Maximal release was determined by solubilization of target cells in 100 µl 0.1 M NaOH.
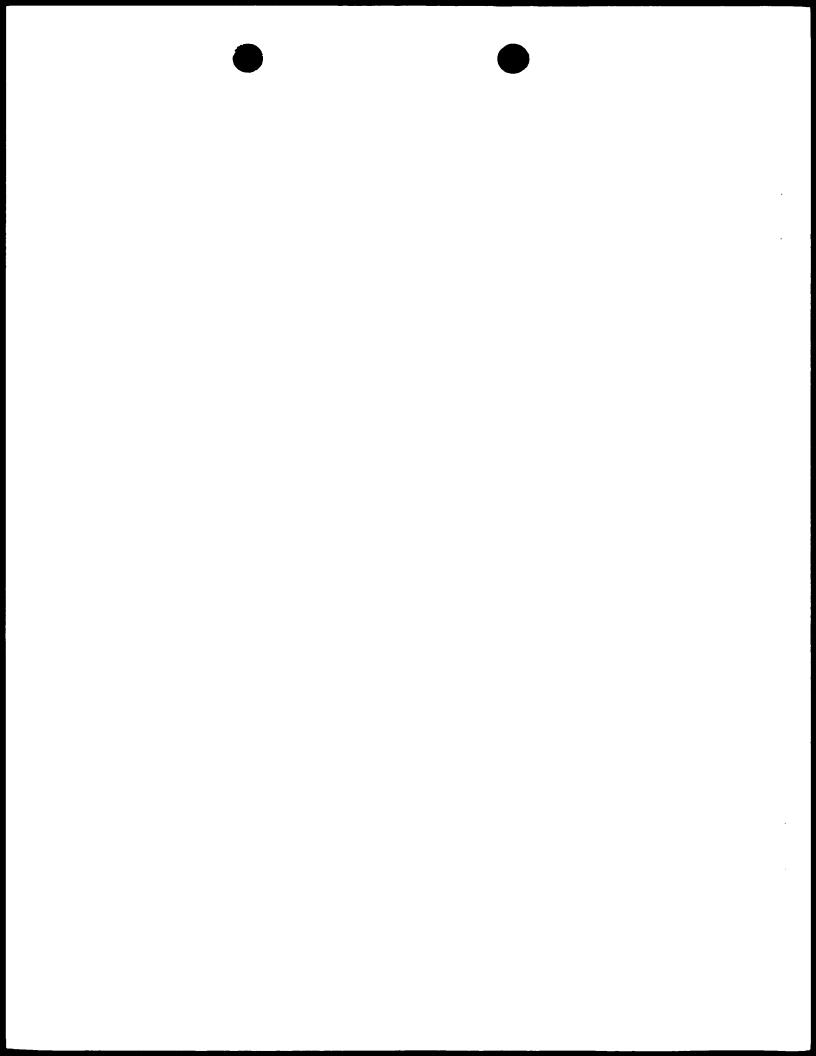
**Sensitization of human PBL *in vitro:*** Patients were HLA typed by the Tissue Typing Unit of The Rabin Medical Center, Israel. Only HLA-A2.1 carrying haplotypes were used herein. Heparanized blood, 40 ml/patient was separated on Ficoll gradients, mononuclear cells were collected at the interphase, washed three times in PBS and resuspended in PBS. A third of the lymphocyte volume was incubated with tumor extracted peptides, at a concentration of 1 $O.D_{230}$/ml for 2 hours at 37°C. The other two thirds of the cells were suspended at $10^6$ cells/ml in RPMI - 10 % FCS, 2 mM glutamine, 1 mM sodium pyruvate, 10 mM Hepes, 5 x $10^{-3}$ M β-mercaptoethanol and combined antibiotics. Peptide loaded cells were added together with 5-20 U/ml of recombinant human IL-2 (Peprotec, Rehovot, Israel). Cells were incubated for 5-7 days at 37 °C, 5 % $CO_2$ and then used in CTL assays as described above.

## EXPERIMENTAL RESULTS

### EXAMPLE 1
### Uroplakins are T cell defined TAAs in transitional cell carcinoma (TCC)

Transitional cell carcinoma (TCC) of the bladder represents a prevalent cancer. Its incidence increases gradually during life and peaks in the 7th and 8th decade to 200/100,000. About 95 % of bladder tumors are TCC. Of these, 75 % are superficial papillary tumors and have a 15 % - 20 % progression rate during the first two years of follow up. Approximately, 20 % - 25 % of TCC present as muscle invasive tumors with aggressive behavior and a 40 % survival rate at 5 years (46). After surgical resection of superficial tumors, intravesical instillations of chemotherapeutics as Tiotepa or Mitomycin, or non-specific intravesical immunotherapy with Bacillus Calmette Guerein (BCG), reduce the frequency of recurrences and have a minimal effect on progression. BCG intravesical instillation was found to be the most effective in reducing the recurrence and progression rate of high grade papillary TCC and of transitional cell carcinoma *in situ*. The mechanism of action was not yet elucidated. However, following therapy there is a mononuclear infiltrate of the lamina propria of the bladder

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mucosa. This infiltrate is rich in T-cells, macrophages and Langerhans cells (47). It was found that CD8 T-cells were activated after BCG intravesical therapy and induced specific lysis of TCC cells (48). Also, BCG intravesical instillation resulted in increased presentation of MHC complexes on transitional cell membrane (49). Lately, CTL, which induced specific lysis of autologous tumor cells, were identified among tumor infiltrating lymphocytes grown from transitional cell carcinomas of the bladder (50). It seems that specific cytotoxic T-cell immunity against TCC may be augmented by BCG intravesical therapy.

An important group of TAAs are actually differentiation antigens. Such antigens were shown to induce CTL in melanoma and in colon carcinoma patients (22, 23).
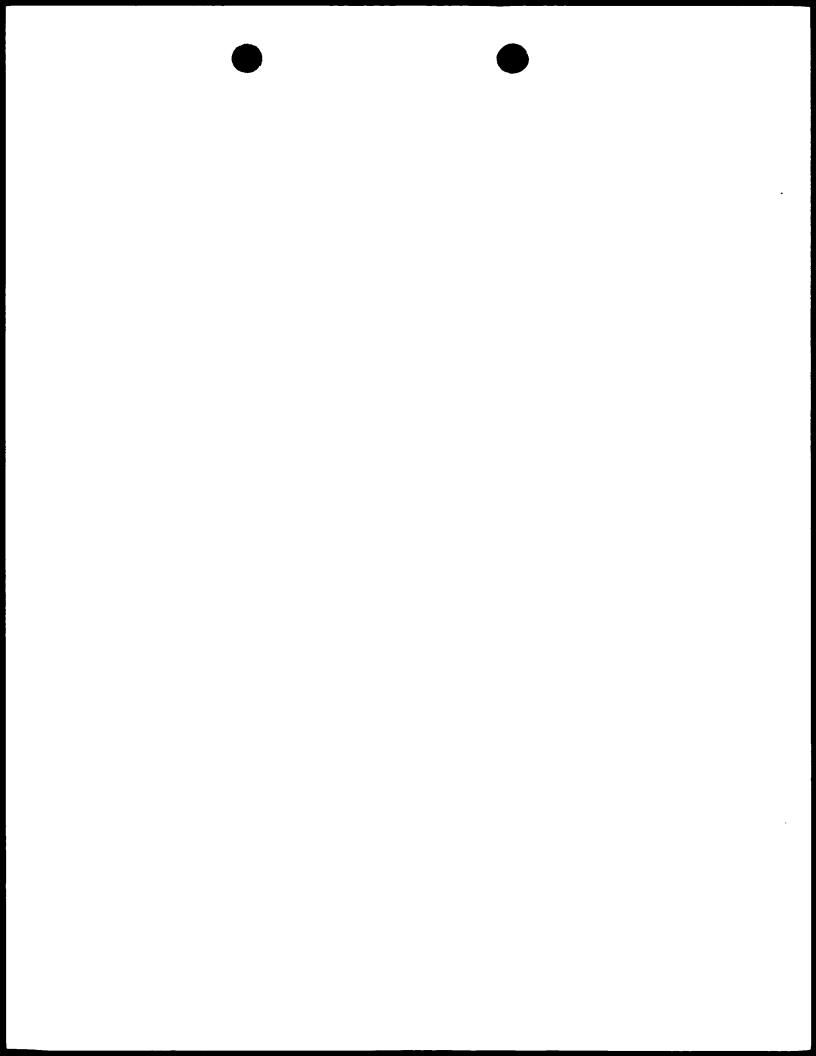
Transitional epithelium is a differentiated multilayered epithelium which is restricted to the urinary tract. It is composed of 4 to 7 cell layers: a basal cell layer; an intermediate 2-3 cell layer and an apical (luminal) layer composed of umbrella like cells. These latter cells are connected by tight junctions and create an impermeable barrier to water and urinary solutes.

Lately, differentiation proteins of transitional epithelium have been characterized morphologically and biochemically. These proteins were found in urothelial plaques which are present in the luminal plasma membrane of urothelial superficial (umbrella) cells. The molecular constituents of these plaques comprise four transmembranal proteins designated Uroplakins (UP); UP Ia (27 Kd); UP lb (28 Kd); UP II (15 Kd) and UP III (47 Kd glycoprotein; the size of the core protein is 28.9 Kd). Uroplakin III probably plays a role in the formation of the urothelial glycocalyx and may interact with the cytoskeleton. An extensive evaluation of UP tissue distribution showed that UP are restricted to normal transitional cell epithelium and to TCC (51). In normal transitional epithelium the UPs were found in the apical membrane of the superficial umbrella cells. In superficial papillary TCC, the UPs were localized in the apical membrane of the luminal cells and in the cell membranes lining intra and inter-cellular lumina. In invasive TCC, UPs were localized more randomly in the cell membrane of the cells which infiltrated the stroma. Recently, the sequence of human Uroplakin II cDNA and Uroplakin Ia genomic sequence were determined and their protein sequences were deduced (NCBI accession Nos. 2190407 and 2098577, respectively). Since TCC patients treated by BCG were shown to have increased T cell infiltrates in the bladder mucosa, we first tested whether BCG treated patients show increased CTL activity against TCC derived peptides and whether UP II peptides may constitute CTL specific epitopes. We further used the TCC

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model to test the T cell repertoire overlap between patient derived CTL and CTL induced in the unique H-2D$^{b-/-}$ x β2m$^{-/-}$ x HhD$^{+/+}$ (HhD) mice.

Cytotoxic activity of human peripheral blood lymphocytes (PBL) against target cells pulsed with Uroplakin II peptide homologues: The human

5  UP II amino acid sequence was screened for potential HLA-A2 binding, 9 amino acids peptides by a HLA binding motif program. We chose that particular HLA allele because it is present in 45 % of the population. Seven 9-mer peptides, which were predicted to bind with high affinity to HLA-A2 were selected and synthesized (Table 1). The binding of these peptides to HLA-A2 was tested by an

10  MHC stabilization assay utilizing TAP negative cells, which can present exogeneously bound peptides. The RMA-S cells (30) transfected by the HhD construct were loaded with various concentrations of UP II peptides and then reacted with anti-HLA antibody. High affinity binding was shown for all 7 peptides by FACS analyses (Figure 1).

15

## TABLE 1
### Predicted human Uroplakin II peptides that bind to HLA-A2
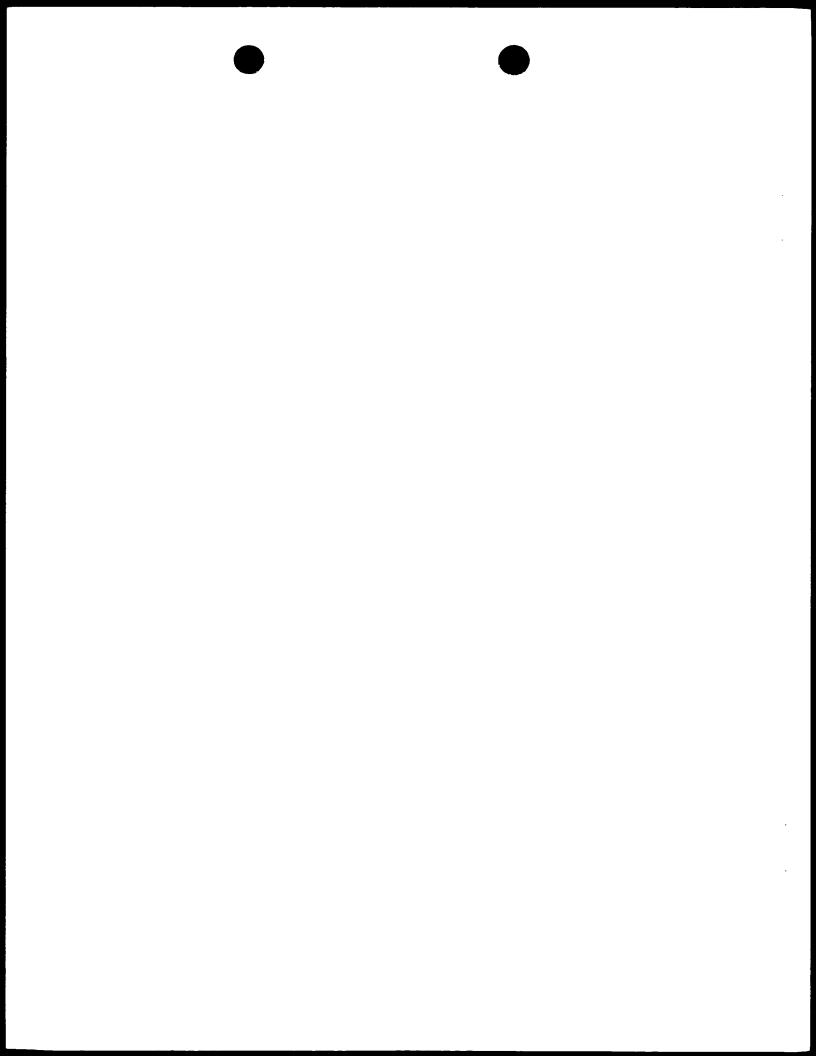
| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---------|----------|--------------|---------|
| 20  HURO1 | FLLVLGFII | 168-176 | 1 |
| HURO2 | VLPSVAMFL | 161-169 | 2 |
| HURO3 | LVLGFIIAL | 170-178 | 3 |
| HURO4 | KVVTSSFVV | 67-77 | 4 |
| HURO5 | LVPGTKFYI | 109-117 | 5 |
| 25  HURO6 | LLPIRTLPL | 4-12 | 6 |
| HURO7 | YLVKKGTAT | 119-127 | 7 |

The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability.

30  Single letter amino acid codes and the position of the peptide in the protein are listed. * Numbering is according to NCBI accession No. 2190407.

To restimulate CTL activity in patient's PBL we chose 6 TCC patients of the HLA-A2 haplotypes: 4 patients underwent adjuvant intravesical BCG

35  instillations and 2 underwent no adjuvant therapy after endoscopic resection of the TCC. Another patient without TCC served as control. PBL were stimulated in vitro by a mixture of tumor acid extracted peptides from 9 TCC samples as described before (52). Each tumor was extracted individually and an equimolar
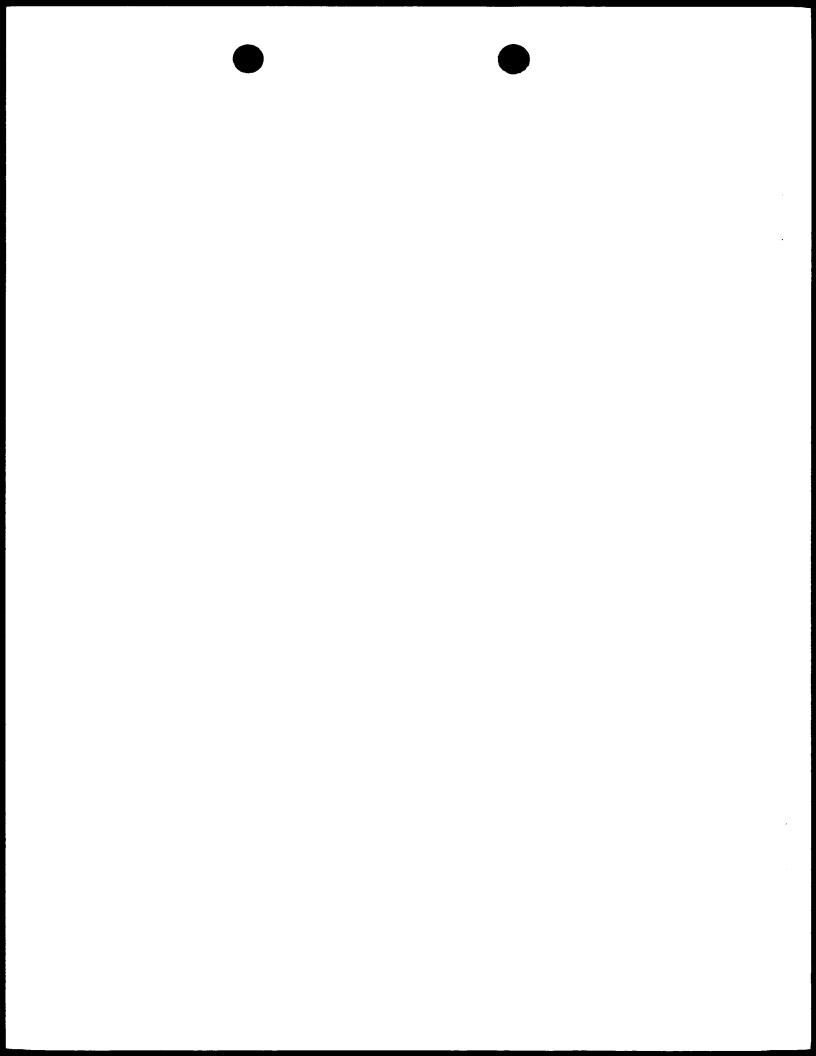
ratio of each sample was added to the mixture. The final concentration of peptides in the stimulation reaction was 1 O.D. 230/ml. Antigen presenting cells (APC) from the same peripheral blood were pulsed with the tumor extract. Peripheral blood lymphoyctes (PBL) were stimulated with APC loaded with pooled TCC

5 extract and with IL2 for 5 days. Uroplakin II peptides, TCC extracted peptides and normal bladder extracted peptides were loaded on labeled T2 cells (HLA-A2 positive cells which are TAP deficient, express empty MHC molecules and can be loaded only with exogenous peptides), and were screened by CTL obtained from PBL of the patients, as described. CTL from TCC patients treated with BCG

10 induced 32 % to 71 % specific lysis of Uroplakin II peptide loaded T2 cells (Figure 2). Tumor extract loaded targets were lysed at 62% while normal bladder extract loaded targets were lysed at 29 % only, indicating preferential recognition of tumor antigens. CTL from TCC patients without BCG adjuvant therapy induced 25 % to 48 % specific lysis of T2 cells loaded Uroplakin II peptides, 29 %

15 lysis of tumor extract loaded T2 cells and 15 % lysis of bladder extract loaded T2 cells. There was no lysis of T2 cells loaded with breast tumor extract, or with a HLA-A2 binding peptide homologue to tyrosinase, a melanoma differentiation antigen. A TCC cell line expressing HLA-A2, J82 was effectively killed while another TCC line SUP, was not lysed. Also, there was no lysis of a natural killer

20 (NK) sensitive cell line (K562). CTL obtained from the patient without TCC induced no specific lysis of tumor extract, or Uroplakin II peptides loaded APC cells. The differences between TCC patients treated with BCG, TCC patients without adjuvant therapy and non-TCC patients were very significant ($P < 0.001$). We also tested the activity of patient's PBL, resensitized by the pool of tumor

25 extracts, against individual tumor extracts. Figure 3 shows higher activity of lymphocytes derived from BCG treated patients than lymphocytes derived from non-treated patients. Control lymphoyctes show low activity. Thus shared antigens are expressed in all TCC samples.

**CTL induced in HhD mice recognize the same UP II peptides as**

30 **human PBL:** The immunogenicity of Uroplakin homologue peptides was also evaluated in a mouse model, in which the murine MHC class I genes are not expressed ($D^{b-/-}$, β2-microglobulin -/-, double knockout) and which is transgenic for a HLA-A2 and human β2-microglobulin monochain (HhD mice). The advantage of a HLA-A2 transgenic mouse model is that CTL epitopes can be

35 detected more easily and reproducibly, without lengthy and repeated *in vitro* stimulations. The murine MHC knockout mice used herein has the additional advantage that their CTL repertoire is only HLA-A2 restricted and the CTL response against human tumor extract or human cell lines is not masked by a

potential xenogeneic response. HhD mice were immunized with the 7 Uroplakin II homologue peptides or with the same TCC peptide extract used for stimulation of patient's PBL. Lymphocytes were obtained from the spleens of these mice and restimulated *in vitro* once with the corresponding peptides.

5     The 7 UP II peptides, that were recognized by human CTL, 8 new UP Ia homologues peptides with high HLA-A2 binding affinity (Table 2), 4 murine UP II derived HLA-A2 binding peptides (Table 3), TCC and normal bladder mucosa peptide extracts and two TCC cell lines: J82 (HLA-A2 positive) and TCCSUP were screened by the CTL obtained from these mice.

10

## TABLE 2

### *Predicted human Uroplakin Ia peptides that bind to HLA-A2*

| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---------|----------|--------------|---------|
| HURO11 | SLFAETIWV | 33-41 | 8 |
| HURO12 | MLIAMYFYT | 248-256 | 9 |
| HURO13 | LMWTLPVML | 241-249 | 10 |
| HURO14 | MLIVYIFEC | 100-108 | 11 |
| HURO15 | YIFECASCI | 104-112 | 12 |
| HURO16 | LVLMLIVYI | 97-105 | 13 |
| HURO17 | ALCRRRSMV | 85-93 | 14 |
| HURO18 | LLSGLSLFA | 28-36 | 15 |
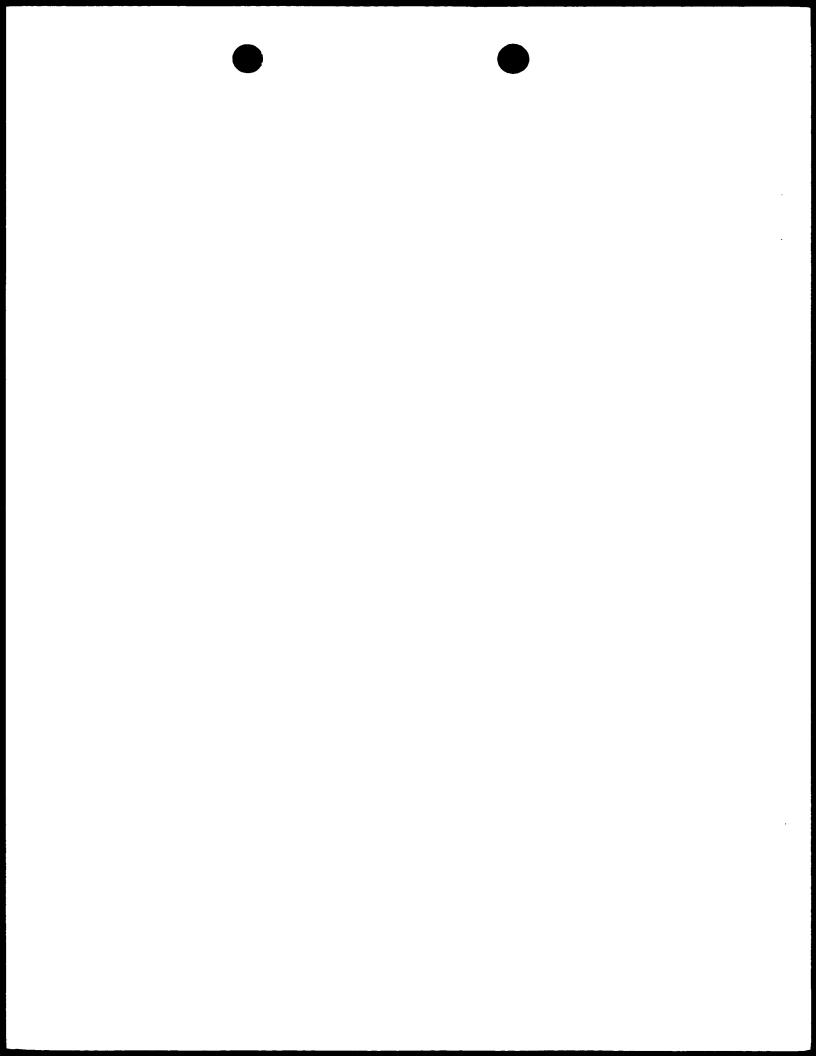
The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the peptide in the protein are listed. * Numbering is according to NCBI accession No. 2098577.

## TABLE 3

### *Predicted murine Uroplakin II peptides that bind to HLA-A2*

| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---------|----------|--------------|---------|
| MURO1 | FLLVVGLIV | 168-176 | 16 |
| MURO3 | LVVGLIVAL | 170-178 | 17 |
| MURO4 | KVVKSDFVV | 69-77 | 18 |
| MURO6 | TLPVQTLPL | 4-12 | 19 |

The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the peptide in the protein are listed. The murine 1, 3, 4, 6 peptides are homologous to the human Uroplakin II

peptides 1, 3, 4, 6 in Table 1. * Numbering is according to NCBI accession No. 586160.

The results are expressed as lytic units (LU30), which represent $10^6$/the number of effectors which induce 30 % specific target lysis. More LU signify a more potent CTL response. Figure 4 shows that CTL from HhD mice immunized with Uroplakin II peptides (1-7 loaded individually and injected by a mixture of loaded cells) induced a significant lysis of RMA-S-HhD cells loaded with Uroplakin II homologue peptides (8.1 to 32.2 LU). There was no lysis of RMA-S-HhD cells loaded with nonspecific peptides as breast tumor extract or the HLA-A2 binding tyrosinase homologue peptide. Also, there was no lysis of RMA-S-HhD cells without peptides (empty target cells). There was intense lysis of J82 cells (17.3 LU) and there was much less lysis of TCCSUP cells (1.2 LU). There was also intense lysis of RMA-S-HhD cells loaded with TCC peptide extract (15 LU) and less lysis of normal bladder mucosa peptide extract loaded cells (4.5 LU) (Figure 4).
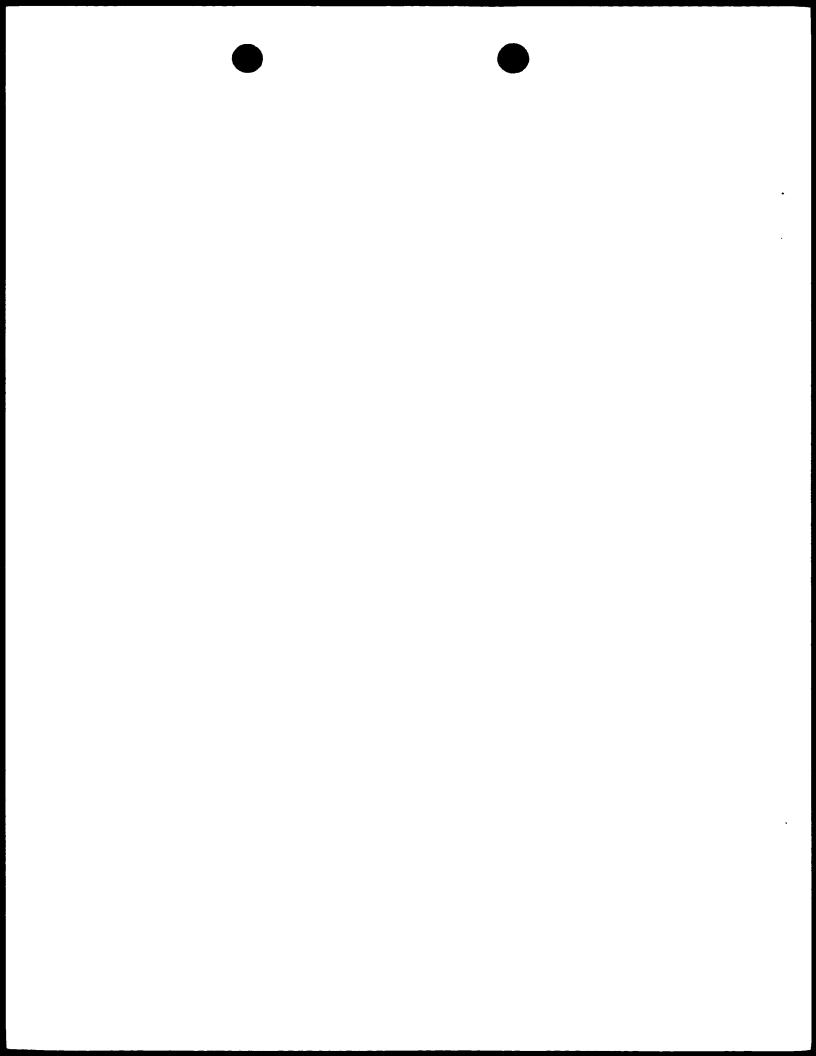
CTL from mice immunized with TCC peptide extract induced significant lysis of RMA-S-HhD targets loaded with Uroplakin II homologue peptides (3.2 to 14 LU); Uroplakin Ia homologue peptides (2 to 11.4 LU); TCC peptide extract (12.6 LU) and less lysis of these targets loaded with normal bladder mucosa peptide extract (2 LU). There was also intense lysis of J82 cells (7.6 LU) and less lysis of TCCSUP cells (0.8 LU). There was no lysis of target cells loaded with nonspecific peptides or of empty target cells. The lysis pattern of the targets loaded with the different Uroplakin II homologue peptides was similar to the CTL assays using human PBL, CTL from HhD mice immunized with Uroplakin II peptides or with TCC peptide extract (Figure 5).

Although human and HhD murine CTL recognize the same UP II peptides, it was not clear whether HhD murine CTL are directed against the actual T cell epitopes or against possible differences between humanoid and rodent UP II. We evaluated also the crossreactivity of CTL from mice immunized with human Uroplakin II peptides against 4 homologue murine Uroplakin II peptides. The murine UP II sequence was screened for HLA-A2 binding peptides and 4 peptide homologues to human peptides 1, 3, 4 and 6 were synthesized. Murine peptides differ in 1-3 amino acids from similar human peptides, yet most are conservative changes (Tables 1 and 3). Murine UP II peptides bind stably to RMA-S-HhD cells.

There was significant lysis of the targets loaded with each of these murine peptides. A good correlation between the LU30 results for the human and their

homologue murine peptides was found. Three human peptides induced more target lysis than their murine counterpart and for the fourth peptide the trend was opposite (Figures 4 and 5). Thus, the specificity of the HhD murine CTL, as the specificity of human PBL is directed to common epitopes on the peptides. To examine whether any autoimmune effects are observed in peptide vaccinated HhD mice, the internal organs of the HhD mice immunized with TCC peptide extract or with Uroplakin II homologue peptides were formalin fixed and representative sections from each organ were stained with hematoxylin and eosin. Two non-immunized HhD mice served as control. There were no pathological finding such as tissue necrosis or inflammatory infiltrate in any of the organ examined. The urinary bladder was carefully examined using a larger number of sections. The bladder wall including the mucosa was normal in the immunized and in the control mice.
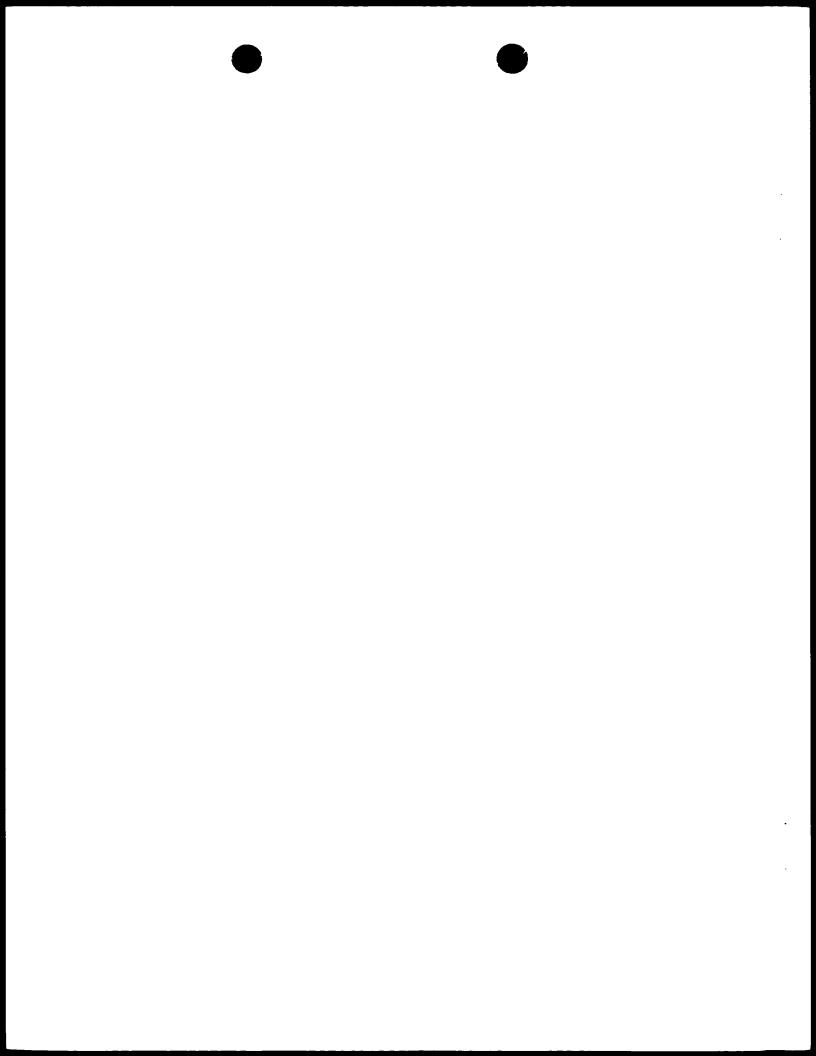
The immunogenicity of additional Uroplakin-derived peptides was evaluated in the HhD mouse strain, in which the murine MHC class I genes are not expressed (Db$^{-/-}$, β2-microglobulin$^{-/-}$ double knock-out), and which is transgenic for an HLA-A2 and human β2-microglobulin monochain. Seven HLA-A2 binding peptides derived from the sequence of Uroplakin Ib, 2 from Uroplakin II, and 6 from Uroplakin III (see Table I) were tested for their ability to evoke specific CTL responses in these mice. After three weekly immunizations, splenic lymphocytes were restimulated *in vitro* with cognate peptide and then incubated with radiolabeled peptide-loaded target cells. Figures 23a-e show that several of the tested peptides (peptides B1, B2, B5, B6, 3.2, 3.3, and 8) could elicit lymphocytes which specifically lysed target cells. Importantly, in all assays there was no significant lysis of unloaded target cells (non) or of cells loaded with a non-specific HLA-A2 binding peptide from the melanoma antigen tyrosinase (tyr).

Table 3a below shows the sequences of the peptides tested in single letter amino acid code, their starting position in the intact protein.
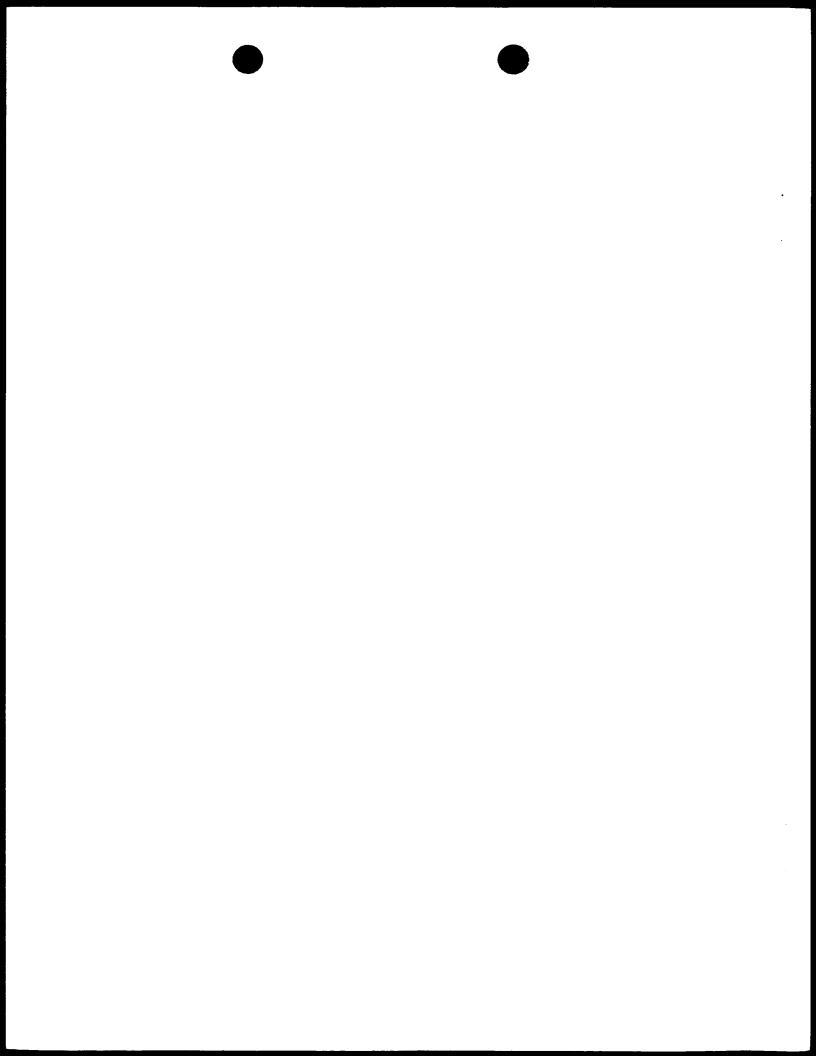
## TABLE 3a
### Predicted human Uroplakin Ib, II and III peptides that bind to HLA-A2

| Peptide | Start Position | Sequence |
|---|---|---|
| Uroplakin Ib/B1 | 239 | AILCWTFWV |
| Uroplakin Ib/B2 | 92 | FILMFIVYA |
| Uroplakin Ib/B3 | 29 | LTAECIFFV |
| Uroplakin Ib/B4 | 154 | MLQDNCCGV |
| Uroplakin Ib/B5 | 240 | ILCWTFWVL |
| Uroplakin Ib/B6 | 86 | KILLAYFIL |
| Uroplakin Ib/B7 | 64 | FVGICLFCL |
| Uroplakin II/8 | 161 | VLLSVAMFL |
| Uroplakin II/9 | 162 | LLSVAMFLL |
| Uroplakin III/3.1 | 214 | ILGSLPFFL |
| Uroplakin III/3.2 | 128 | ILNAYLVRV |
| Uroplakin III/3.3 | 221 | FLLVGFAGA |
| Uroplakin III/3.4 | 20 | NLQPQLASV |
| Uroplakin III/3.5 | 47 | CMFDSKEAL |
| Uroplakin III/3.6 | 62 | YLYVLVDSA |
| Tyrosinase | 368 | YMDGTMSQV |

Table 3a shows the sequences of the peptides tested in single letter amino acid code and their starting position in the intact protein (according to NCBI accession nos. 3298345 (peptides 3.1-3.6), 3483011 (peptides 8 and 9), and 3721858 (peptides B1-B7).

## EXAMPLE 2
### PSA, PSMA and PAP homologue peptides are immunogenic CTL epitopes in HLA-A2 transgenic H-2D$^b$, β2m double knockout mice

Carcinoma of the prostate (CAP) is the most prevalent cancer and the second cause of death in man (53). The use of prostate specific antigen (PSA) in early detection of CAP has caused a stage shift of the disease (54). By now, 70% of CAP cases are detected when they are still organ confined. These patients may be cured by radical prostatectomy or by radiation therapy. However, the long term cancer specific survival of organ confined disease after radical prostatectomy, even in the best series, is only 70 % (55). Patients with large tumors, high grade tumors and those with seminal vesicle invasion, or positive lymph nodes are at increased risk of metastases and death due to CAP (56). However, CAP is a slow growing tumor and there is no effective adjuvant chemotherapy (57). Androgen ablation by surgical or medical means does not

increase survival, but only increases time to recurrence and palliate symptomatic bone metastases (58). There is a need for an effective adjuvant or neo-adjuvant therapy for CAP and for systemic therapy for metastatic CAP. Thus, specific immunity is being investigated as a potential adjuvant therapy. PSA and prostate
5 specific membrane antigen (PSMA) are proteins which are expressed almost exclusively in prostate tissue, benign or malignant and are differentiation antigens. Prostate acid phosphatase (PAP) is expressed also in other tissues but its concentration is much higher in prostate tissue. In a few studies, 3 PSA homologue peptides (59-60) and 2 PSMA homologue peptides (61-62) were found
10 to be immunogenic and to induce specific CTL by repeated *in vitro* stimulation of peripheral blood lymphocytes from CAP patients. In one of these studies (62), patients with metastatic prostate cancer were immunized with the 2 immunogenic PSMA peptides, loaded on dendritic cells. There was some partial responses expressed by a reduction in PSA levels and some objective reduction in bone
15 metastases burden. However, the procedures used to induce CTL lines *in vitro* are cumbersome, lengthy and not reliable. Some CTL peptide epitopes which bind with high affinity to MHC class I may induce tolerance and will be not detected. Moreover, repeated *in vitro* stimulations, necessary for inducing CTL lines may select CTL which have a growth advantage in culture and not the most potent and
20 specific CTL *in vivo*. Herein the HLA-A2 transgenic, H-2D$^{b-/-}$ $\beta$2m$^{-/-}$ double knockout mice (HhD mice) was used for identification of novel prostate cancer specific CTL epitopes. Peptides homologue to PSA, PSMA and PAP, which are expressed mainly in benign or malignant prostate tissue, were evaluated for their capacity to induce specific CTL in HhD mice. The amino acid sequences of these
25 proteins (NCBI accession Nos. 296671, 2897946 and 439658) were screened by a HLA binding motif program. The peptides which were predicted to bind to HLA-A2 were synthesized (Tables 4, 5, 6) and their binding affinity was tested on RMA-S-HhD cells (TAP deficient cells, capable of presenting only exogenous peptides and which were transfected with the HhD construct) by FACS (Figures 6
30 and 7). The peptides with high binding affinity were used for immunization of HhD mice. Five PSA homologue peptides, in addition to the peptides detected by other authors, 6 PSMA homologue peptides, including the 2 peptides detected by other authors (PSMA 5 and 6) and 4 PAP homologue peptides were found.
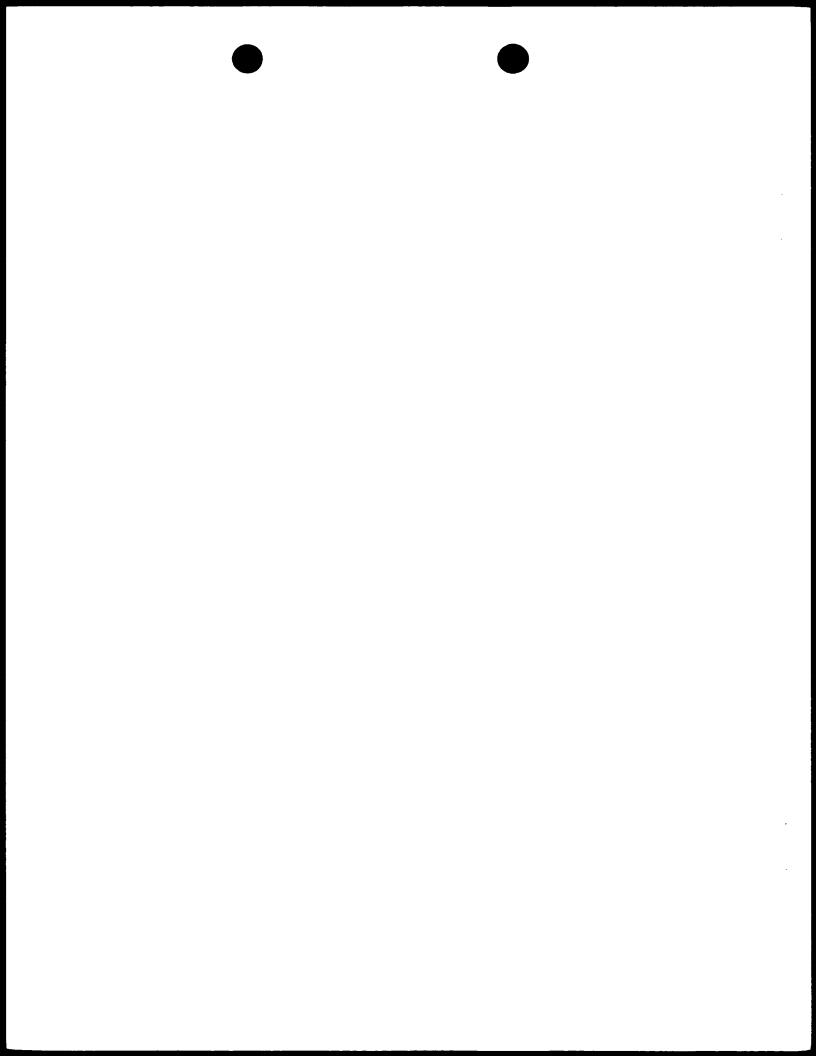
35
## TABLE 4

*Predicted human prostate specific antigen (PSA) peptides that bind to HLA-A2*

| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---------|----------|--------------|---------|
| PSA1 | DLHVISNDV | 175-183 | 20 |

| | | | |
|---|---|---|---|
| PSA2 | VLVHPQWVL | 53-61 | 21 |
| PSA3 | FLRPGDDSS | 110-118 | 22 |
| PSA4 | ALGTTCYAS | 147-155 | 23 |
| PSA5 | KLQCVDLHV | 170-178 | 24 |

The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the peptide in the protein are listed. * Numbering is according to NCBI accession No. 296671.

## TABLE 5

*Predicted human prostate specific membrane antigen (PSMA) peptides that bind to HLA-A2*
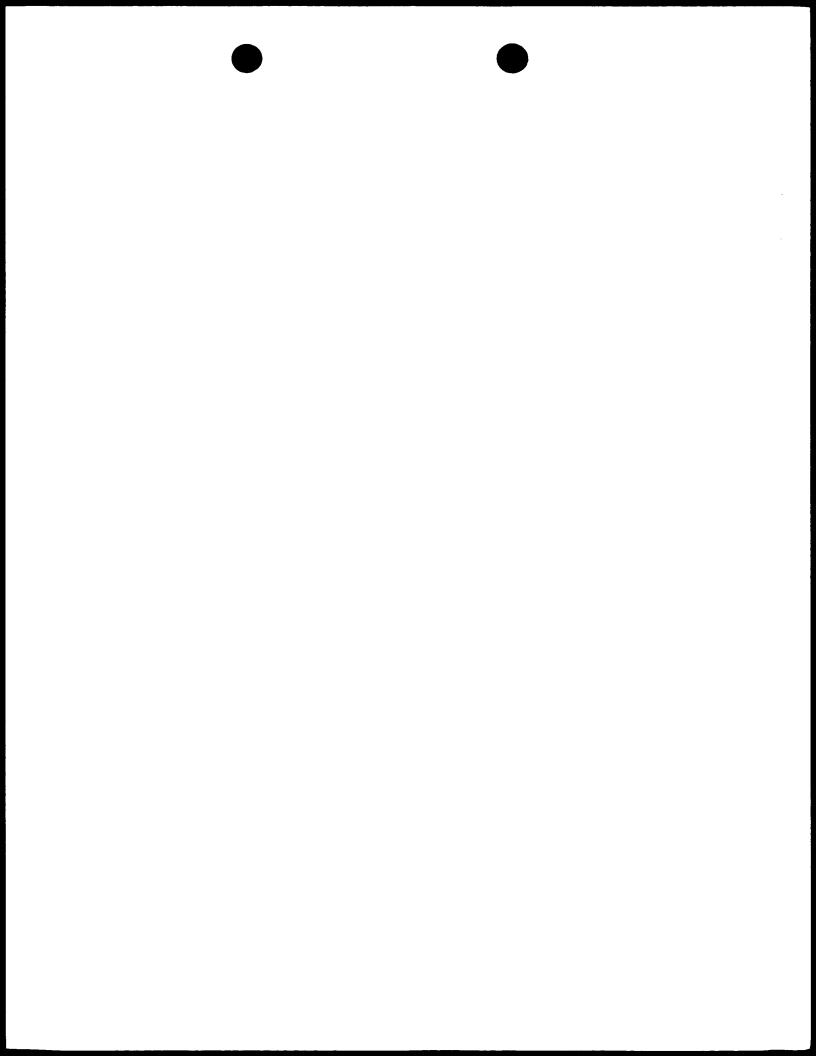
| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---|---|---|---|
| PSMA1 | ELAHYDVLL | 109-117 | 25 |
| PSMA2 | NLNGAGDPL | 260-268 | 26 |
| PSMA3 | TLRVDCTPL | 461-469 | 27 |
| PSMA4 | MMNDQLMFL | 663-671 | 28 |
| PSMA5 | ALFDIESKV | 711-719 | 29 |
| PSMA6 | LLHETDSAV | 4-12 | 30 |

The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the peptide in the protein are listed. * Numbering is according to NCBI accession No. 2897946.

## TABLE 6

*Predicted human prostate acid phosphatase (PAP) peptides that bind to HLA-A2*

| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---|---|---|---|
| PAP1 | VLAKELKFV | 30-38 | 31 |
| PAP2 | ILLWQPIPV | 135-143 | 32 |
| PAP3 | DLFGIWSKV | 201-209 | 33 |
| PAP4 | PLERFAELV | 352-360 | 34 |

The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the peptide in the protein are listed. * Numbering is according to NCBI accession No. 439658.

HhD mice were immunized and boosted twice with the PSA, PSMA, or PAP peptides. Peptides were loaded individually on RMA-S-HhD cells and equal numbers of pulsed cells were mixed in the vaccine. Mice were also immunized with CAP peptide extract, or with DU145-HhD, a CAP cell line transfected with the HhD construct. Lymphocytes were obtained from the spleen of these mice and restimulated once, *in vitro* with the corresponding peptide tumor extract or cells. PSA, PSMA, PAP, CAP and normal prostate peptide extract and DU145-HhD cells were screened by the CTL obtained from these mice. The results are expressed as lytic units (LU) which represent $10^6$/Number of CTL effectors which induce 30 % specific target lysis. More lytic units signify a more potent CTL response.

CTL from HhD mice immunized with PAP peptides induced intense lysis of RMA-S-HhD cells (targets) loaded with PAP homologue peptides (20 to 65 LU, Figure 8). Three out of 5 PSA homologue peptides induced significant and specific lysis of targets loaded with the corresponding peptide (5.2 to 11.4 LU, Figure 9). Four out of 6 PSMA homologue peptides induced significant lysis of the targets loaded with the corresponding peptide (5 to 17 LU, Figure 10). Two out of these 4 peptides were found to be immunogenic by other authors, by *in vitro* stimulation of human peripheral blood lymphocytes. CTL obtained from each group of immunized HhD mice induced significant lysis of targets loaded with CAP peptide extract (4.5 to 22 LU). There was significantly less lysis of normal prostate peptide extract loaded targets than of the CAP peptide extract loaded targets for each immunizing peptide (Figures 8, 9 and 10). Also, there was intense lysis of DU-145-HhD cells by CTL obtained from HhD mice immunized with these cells (Figure 11), or immunized by PAP homologue peptides, or CAP peptide extract (28, 19 and 11 LU, respectively, Figure 12). There was no significant lysis of DU145-HhD cells by CTL derived from HhD mice immunized with PSA, or PSMA homologue peptides. These findings result probably from low expression of PSA and PSMA by DU145 cells.

There was good cross reactivity between CTL derived from mice immunized with CAP peptide extract and PSA, PSMA and PAP homologue peptides, which were found to be immunogenic in HhD mice. The CTL derived from mice immunized with DU145-HhD cells cross reacted with the immunogenic PAP homologue peptides (11 to 26 LU) CAP peptide extract (12 LU), and less with normal prostate peptide extract (3 LU). Overall, there was no lysis of targets loaded with nonspecific peptides such as breast cancer peptide extract, or HLA-A2 binding tyrosinase homologue peptide. Also, there was no lysis of RMA-S-HhD cells without peptides and there was no lysis of non transfected DU 145 cells
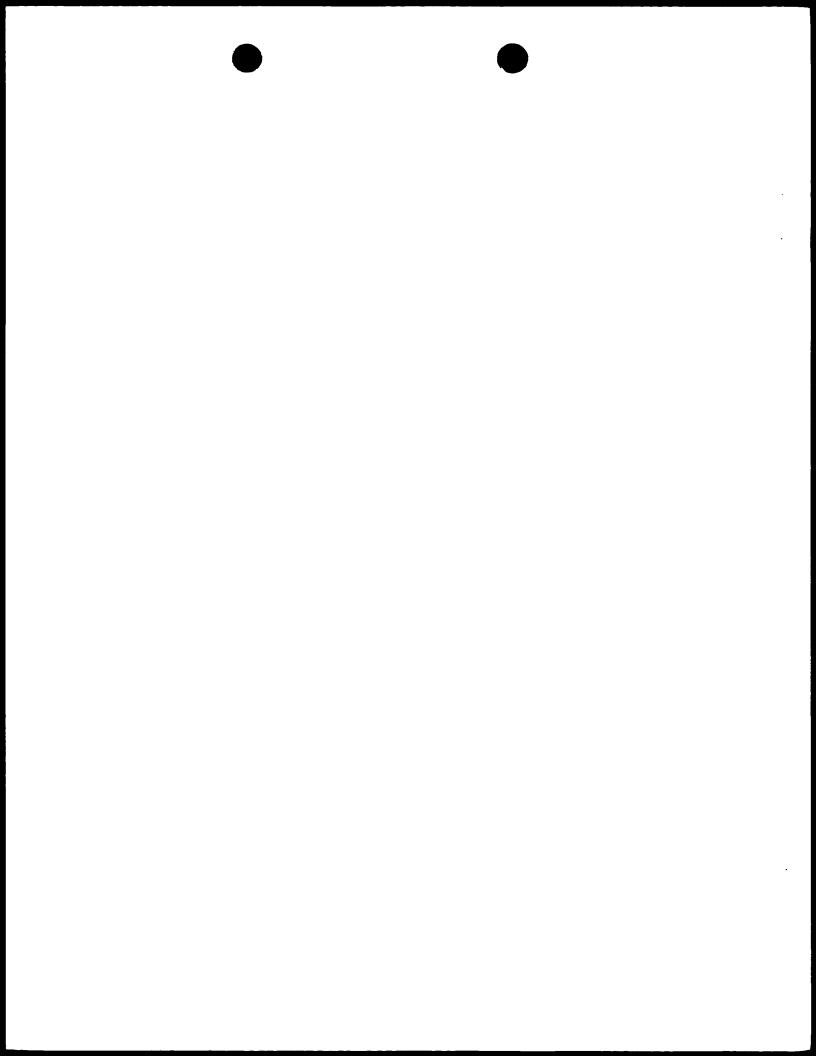
(HLA-A2 negative).  The internal organs of the HhD mice immunized with PSA, PSMA, PAP homologue peptides, or with CAP peptide extract, or with DU145-HhD cells, were formalin fixed and representative slices from each organ were stained with hematoxyllin and eosin.  Two non-immunized HhD mice served as control.  There was no pathological finding such as tissue necrosis or inflammatory infiltrate in any of the organ examined.

## EXAMPLE 3
### Breast specific CTL induction by BA-46 (Lactadherin) peptides and by MUC1 peptides in HhD mice

5       Breast cancer is the second leading cause of cancer death among women in the Western World and the leading cause of death among women at the age of 30 to 70. Breast cancer afflicts 200,000 women per annum in the USA today. The highest mortality is restricted to patients whose regional lymph nodes are involved. Early detection, followed by surgery provides good prognosis. In 10 patients with occult lymph node metastasis, adjuvant chemotherapy or hormonal therapy for breast cancer have been proven to be effective, yet a large fraction of patients will succumb to metastasis. (63).
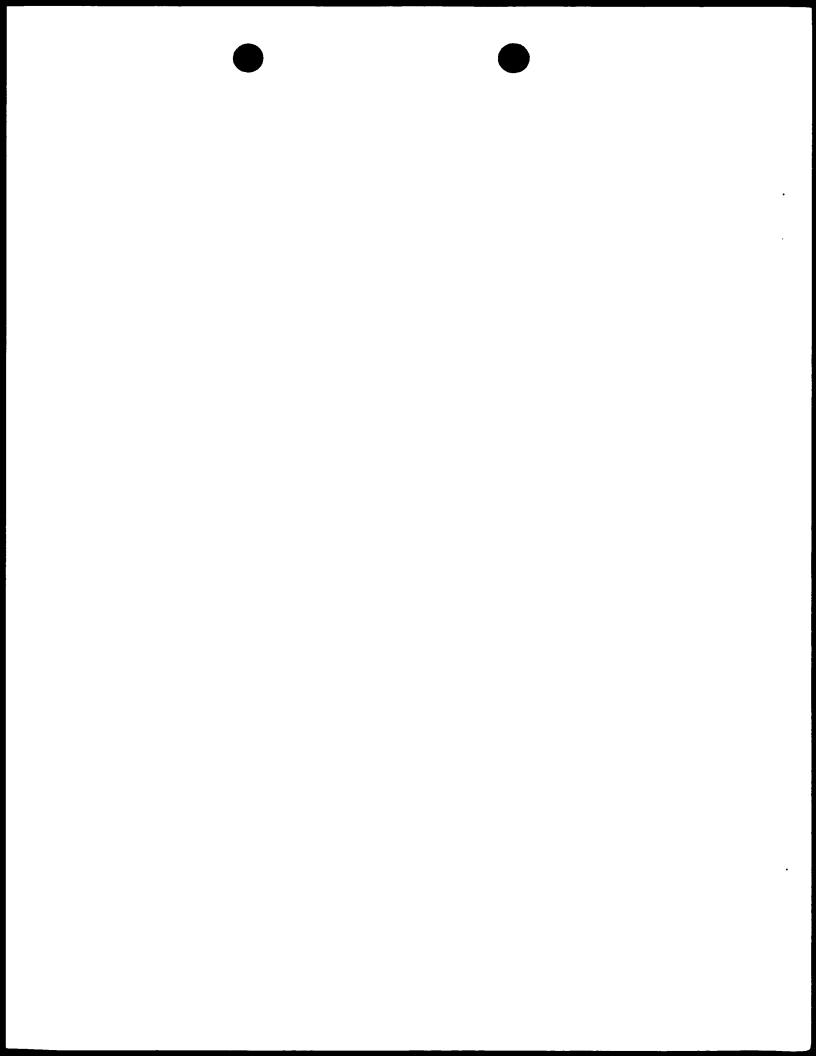
As pointed out above, TAA vaccines may constitute an additional treatment modality for residual disease. A number of tumor associated antigens have been 15 described for breast carcinomas. The MUC-1 Mucin, a high molecular weight glycoprotein is highly expressed on breast carcinomas. Specific, MHC-unrestricted recognition of MUC-1 by human cytotoxic T cells were demonstrated (64). More recently peptides from MUC-1 were shown also to induce MHC class I and MHC class II restricted responses (65, 66). HER2/neu 20 derived peptides were shown to be recognized by CTL from breast carcinoma patients (67).

BA-46 is a 46 kDa transmembrane-associated glycoprotein of the human milk fat globule membrane (HMFG), that is overexpressed in human breast carcinomas (68). The protein contains cell adhesion sequences (RGD), supports 25 RGD based adhesion and interacts with integrin (69). It also contains an EGF-like domain, and a phospholipid binding sequence C1/C2-like domain of coagulation factor V and VIII. BA-46 is present in the circulation of breast cancer patients but not in healthy individuals (70). Moreover, anti BA-46 radio-conjugated monoclonal antibodies, have successfully targeted human breast tumors 30 transplanted into mice (71). Herein we tested MUC1 and BA-46 derived peptides as potential TAA peptides in the HhD mouse system. BA-46 homologous peptide vaccines induce HLA-A2 restricted CTL which preferentially recognize breast tumor derived peptides.

BA-46 is overexpressed in many breast carcinomas, yet, no cellular 35 immunity to BA-46 protein has been reported so far. We evaluated peptides complementary to the amino acid sequences of human BA-46, The sequence was screened for HLA-A2 binding motifs by a HLA binding motif program. Seven

9-mer peptides which were predicted to bind with high affinity to HLA-A2 were selected and synthesized (Table 7).
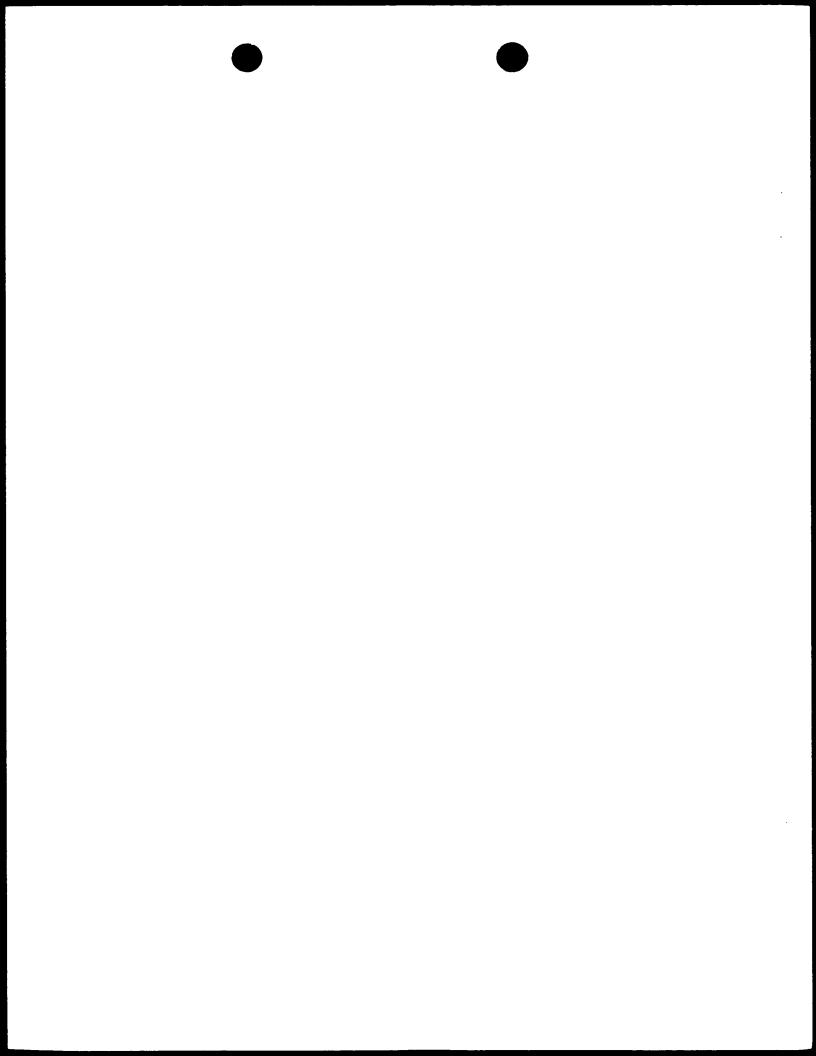
## TABLE 7

*Predicted human breast associated BA-46 peptides that bind to HLA-A2*

| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---------|----------|--------------|---------|
| BA-46-1 | KQGNFNAWV | 271-279 | 35 |
| BA-46-2 | NLLRRMWVT | 131-139 | 36 |
| BA-46-5 | NLFETPILA | 356-364 | 37 |
| BA-46-6 | NLFETPVEA | 194-202 | 38 |
| BA-46-7 | GLQHWVPEL | 97-105 | 39 |
| BA-46-8 | VQFVASYKV | 313-321 | 40 |
| BA-46-9 | RLLAALCGA | 5-13 | 41 |

The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the first amino acid of the peptide in the protein sequence as well as the calculated binding score are listed. * Numbering is according to NCBI accession No. 1589428.

All peptides bound well to the HhD molecules expressed on RMA-S transfectant (Figure 13). HhD mice were vaccinated three times at weekly interval using $2x10^6$ RMA-S-HhD/B7.1 with either tumor extract prepared from 5 samples of breast carcinoma by acid extraction and separation of molecules < 3 Kd, or with a pool of BA-46 peptides. Ten days after the last immunization, HhD spleen-derived antigen presenting cells (APC) were pulsed for 3 hour at 37°C with either breast tumor extract or a BA-46 peptide pool, followed by incubation with the rest of the splenocytes for 4 more days. BA-46 peptides, breast extracted peptides and normal breast extracted peptides were loaded on labeled RMA-S-HhD cells and were screened by cytotoxic T lymphocytes (CTL) obtained from the HhD mice as described before (62).

Anti BA-46 peptide CTL activity showed variability in immunogenicity at a range between 20 % to 60 %. Lysis of 60 % was obtained against BA-46-7, 40 % against BA-46-6, BA-46-9 and 20 % against the rest of the peptides (Figure 14). CTL from HhD mice immunized with breast tumor extract peptide induced 10% to 30 % specific lysis of BA-46 peptide loaded RMA-S-HhD cells, 31 % lysis of tumor extract loaded targets and only 12 % and 14 % lysis of normal breast extracted peptides and colon extracted peptides loaded RMA-S-HhD cells, respectively. No lysis of the melanoma associated synthetic tyrosinase peptide

40

was obtained (Figure 15). The ability of BA-46 peptides to induce a breast associated CTL reaction that is HLA-A2.1 restricted was further examined. CTL against individual BA-46 peptides showed 30-50 % higher activity against breast tumor extract versus normal breast loaded target cells, supporting the fact that a
5    preferential activity is obtained against breast tumor TAAs. These effectors also lysed preferentially a breast carcinoma line-HhD transfectant relative to parental cells, stressing the HLA-A2 restriction of the reaction (Figures 16 and 17).


## EXAMPLE 4

10   **Characterization of novel breast carcinoma MUC1 derived peptides as**
**CTL epitopes**


The polymorphic epithelial Mucin, encoded by the MUC-1 gene is a high molecular weight transmembranal glycoprotein overexpressed in a broad range of
15   tumors, such as breast, pancreas, ovary, thyroid and myeloma (72) cancer. It was found that the growth rate of primary breast tumors induced by the polyoma middle T antigen is significantly slower in MUC-1 null mice, suggesting that MUC-1 might play a role in the progression of mammary carcinoma (73). Furthermore, high level of MUC-1 expression by human breast cancer cells was
20   found to be directly correlated with the presence of axially lymph node metastasis (74). A major feature of the MUC-1 molecule is the presence of an highly immunogenic extracellular tandem repeat array (TRA) heavily O-glycosidic-linked serine and threonine residues (75). Altered carbohydrate structure of MUC-1 in breast cancer cells, is probably responsible for the exposure
25   of core epitopes within MUC-1, specifically recognized by monoclonal antibodies, as well as non MHC-restricted cytotoxic T lymphocytes (76). HLA-A11 (77) and more recently for HLA-A2.1 (78) restricted responses to the extracellular TRA have also been reported. However it was suggested that Mucin can actively suppress cell-mediated response against glycosylated TRA (79), or induced direct
30   T cell apoptosis (80). More recently Agrawal et al showed that synthetic peptides derived from MUC-1 TRA cause suppression of human T-cell proliferative responses (81). This data points out the ambiguous role of MUC-1 TRA in T cell activation.

A series of peptides derived from non-TRA domains of the MUC-1 as
35   potential CTL epitopes. The MUC-1 sequence was screened for potential HLA-A2.1 binding peptides (Table 8) and the eight peptides were synthesized.

41
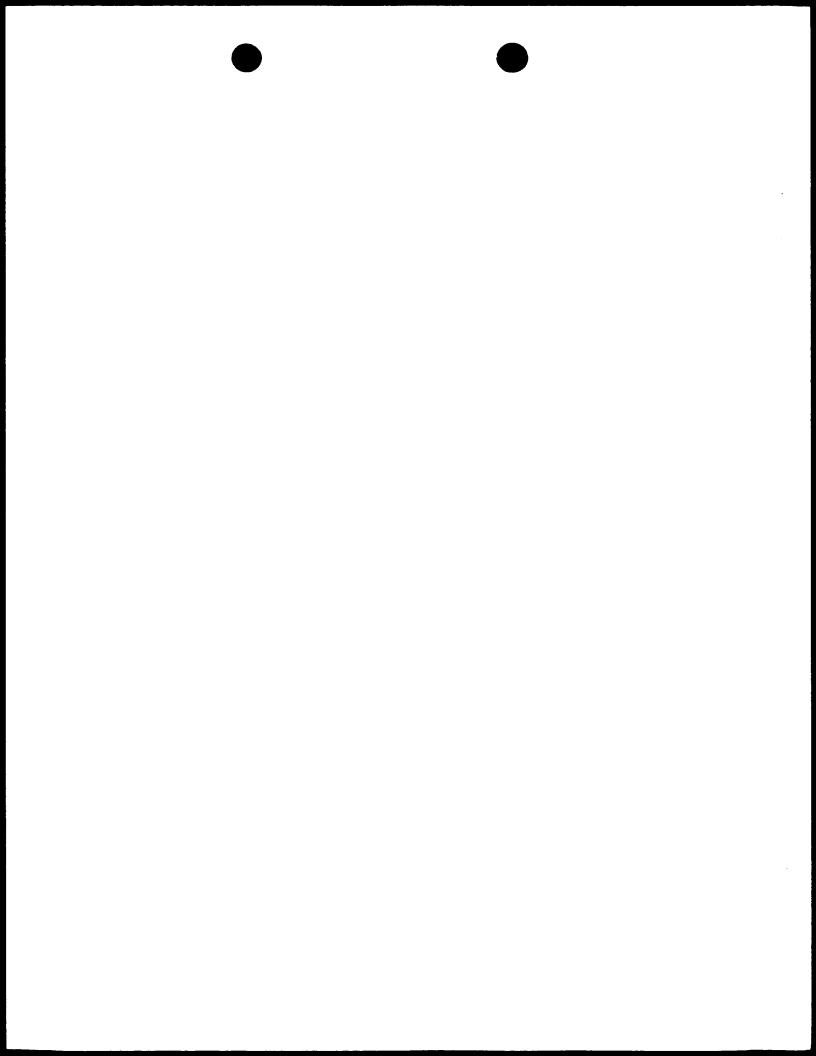
## TABLE 8

### *Predicted human breast associated MUC-1 peptides that bind to HLA-A2*

| Peptide | Sequence | Amino-acids* | SEQ ID: |
|---------|----------|--------------|---------|
| MUC1-/C6 | LLLLTVLTV | 31-40 | 42 |
| MUC1-/D6 | LLLTVLTVV | 32-41 | 43 |
| MUC1-/F6 | FLSFHISNL | 323-331 | 44 |
| MUC1-/A5 | LLVLVCVLV | 442-451 | 45 |
| MUC1-/F4 | ALLVLVCVL | 441-450 | 46 |
| MUC1-/B5 | SLSYTNPAV | 519-528 | 47 |
| MUC1-/A7 | NLTISDVSV | 412-421 | 48 |
| MUC1-/E6 | ALASTAPPV | 226-234 | 49 |
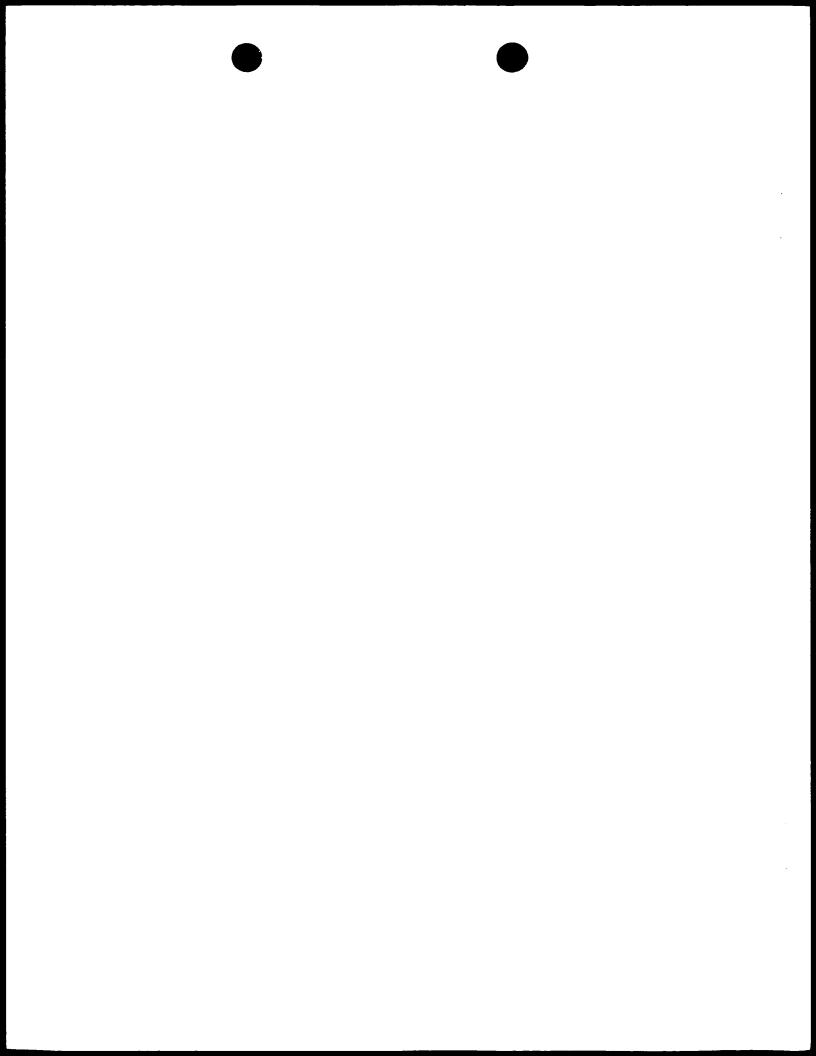
The prediction program is described in Materials and Methods. The peptides are listed in descending order of predicted HLA-A2.1-peptide complex stability. Single letter amino acid codes and the position of the first amino acid of the peptide in the protein sequence as well as the calculated binding score are listed. * Numbering is according to NCBI accession No. 182253.

These 9 residue peptides are derived from the signal peptide, cytoplasmic and extracellular domains of the MUC-1 protein. Among these peptides only the MUC-1/B5 peptide has an identical sequence to the murine homologue. HLA-A.21 binding of MUC-1- derived peptides were evaluated by FACS analysis. The selected peptides were loaded on the murine TAP-deficient RMA-S-HhD transfectants and MHC stabilization was monitored (Figure 18). Although all peptides bound efficiently at the 1-100 µM range, 3 peptides MUC-1/D6, MUC-1/E6 and MUC-1/A7 exhibited higher binding affinity. In addition, similar binding affinities of these peptides were obtained upon loading on human TAP deficient T2 cells, expressing endogenous HLA-A2.1 molecules (data not shown).

Synthetic peptides corresponding to the MUC-1 TRA epitopes were shown to induce CTL reaction in patients (77) as well as in HLA-A2.1K[b] transgenic mice (78). Hence we primarily examined the lysis pattern of each of the particular peptides listed in Table 8 following an immunization with a pool of the MUC-1-derived synthetic peptides (Figure 19). CTL results showed significant lysis of RMA-S-HhD target cells loaded either with the MUC-1/D6 peptide (38 %) or with the MUC-1/A7 peptide (30 %). Lower lysis rate of 15 % with the MUC-1/E6 and with the MUC-1/F6 peptides was demonstrated. The rest of the peptides showed only background lysis properties. Hence MUC-1/D6, MUC-1/A7 and MUC-1/E6 harbor immunogenic properties.

42

To determine the processing and presentation of MUC-1-derived peptides by breast carcinoma tumors, we selected the MDA-MB-157 cell line which is characterized by high MUC-1 expression and low class I expression (data not shown). Both the parental tumor cell line and its HhD transfectant
5 (MDA-MB-157-HhD), were used as targets in CTL assays (Figure 20). Mice were immunized with either RMA-S-HhD-B7.1 cells loaded with MUC-1 selected peptides MUC-1/D6, MUC-1/A7 and MUC-1/E6, or with peptide extract derived from fresh patients' tumors. Preferential lysis of the MDA-MB-157-HhD cell line by anti MUC-1-derived peptides activated lymphocytes suggested both breast
10 associated as well as MHC-restricted lysis. Moreover, this data strongly support the processing and presentation of non TRA associated MUC-1-derive peptides in MDA-MB-157-HhD breast carcinomas cells. Further analysis showed inhibition of lysis by anti HLA monoclonal antibody w6/32 (data not shown). In addition specific lysis of MDA-MB-157-HhD cells by CTL directed against fresh tumor
15 extracted peptides emphasized an overlap in the peptide repertoire between the breast tumor cell line and fresh breast tumor extract.
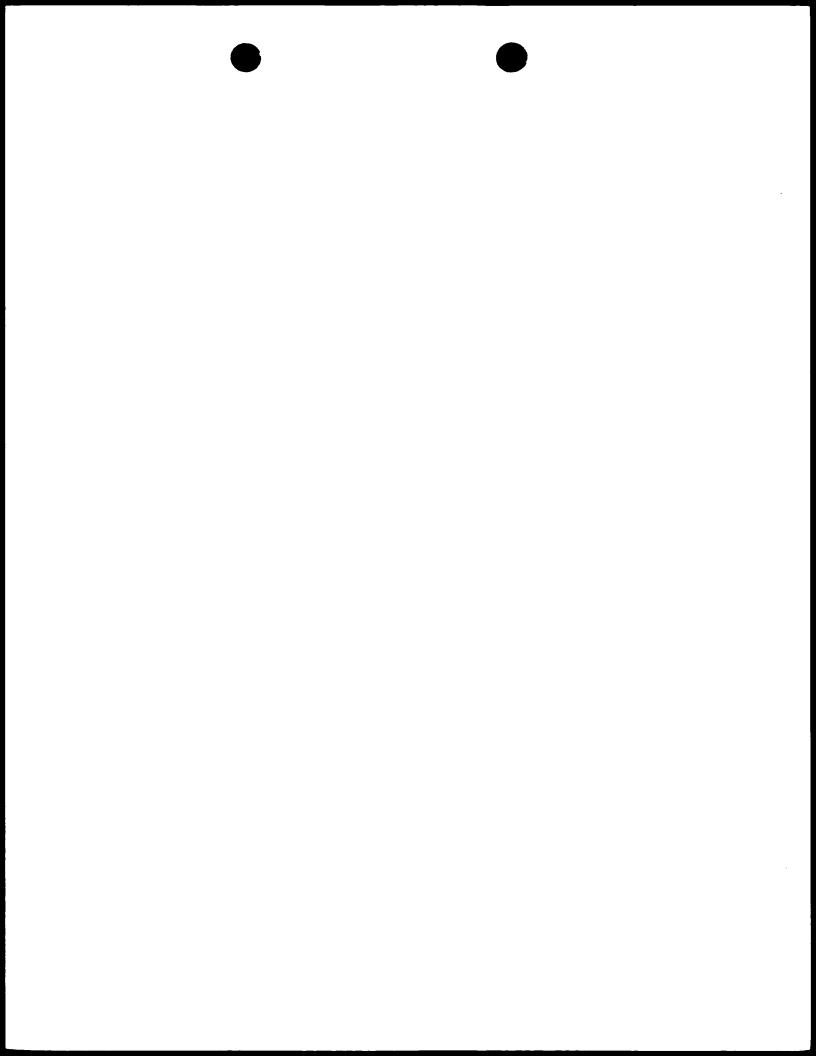
To prove the existence of MUC-1/D6, MUC-1/A7 and MUC-1/E6 peptides in patients' derived breast tumor peptide extracts, a CTL experiment was performed by utilizing CTL against breast tumor peptide extracts as effectors
20 against target cells presenting MUC-1 peptides or a control of normal breast tissue (Figure 21). All 3 MUC-1-derived peptides, but not the HLA-A2.1 melanoma associated peptide tyrosinase, could be recognized and lysed by anti-tumor extract CTL. RMA-S-HhD cells loaded with normal breast extract gave in different experiment between 40-50 % of the lysis induced against tumor extract loaded
25 targets. This result is expected since a part of the tumor extract peptide repertoire consists of normal peptides.

A crucial parameter for selection of TAA peptides based vaccines are their expression frequency by tumors in comparison with normal tissues. Since MUC-1 protein is known to be overexpressed in tumors, with no tumor specific mutations,
30 it is of obvious interest to examine the abundance of MUC-1 peptides in patients' derived normal breast tissue extract in comparison to tumor extract. CTL generated against MUC-1 peptides MUC-1/D6, MUC-1/A7 and MUC-1/E6, showed a 1.8-9.0 fold higher reactivity to tumor extract versus normal tissue extract (Figure 22). The same difference in preferential tumor versus normal
35 tissue recognition by CTL could be detected upon vaccination with breast tumor extract peptides, supporting the window of specificity between normal and tumor tissues. These results suggest that MUC-1/D6, MUC-1/A7 and MUC-1/E6 are potential tumor associated antigen peptides.

43

## EXAMPLE 5

### Induction of CTL response by Teratocarcinoma-derived growth factor (CRIPTO-1) derived peptides.
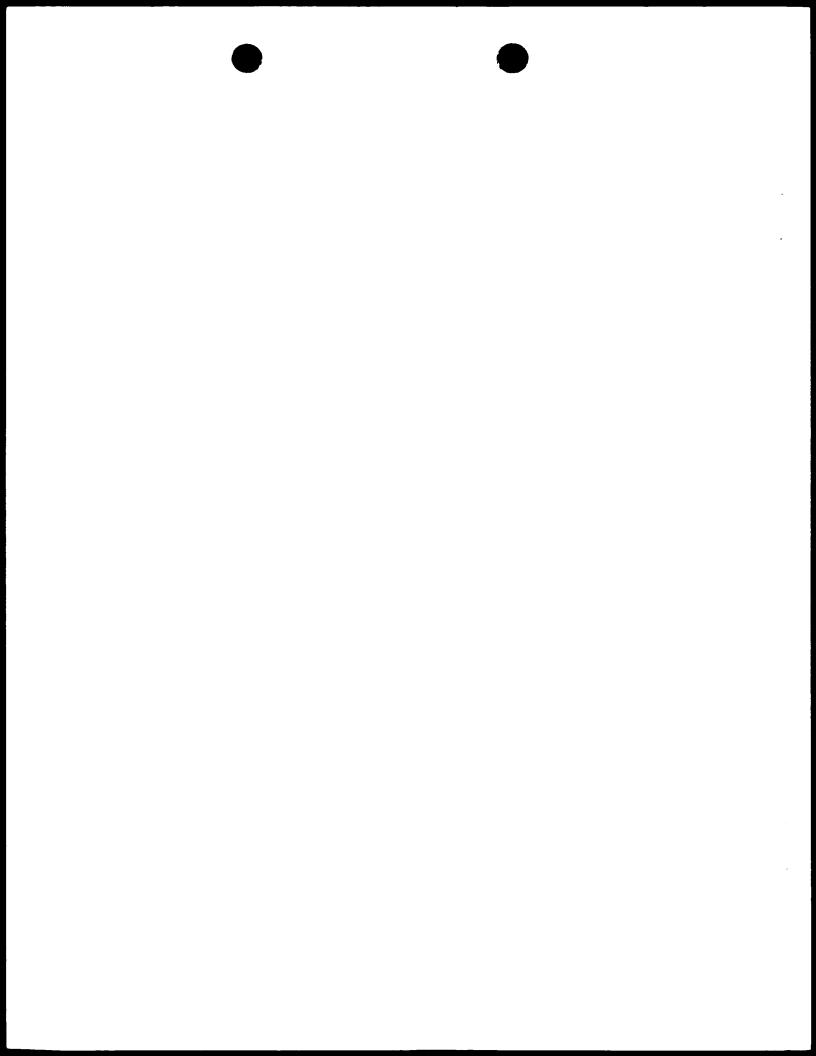
The CRIPTO-1 gene (also known as Teratocarcinoma-derived growth
5    factor) was first identified and cloned from an undifferentiated human embryonal
carcinoma cell line (83). It encodes a 188 amino acid glycoprotein with a region
of structural homology to members of the epidermal growth factor family, but
lacks a hydrophobic signal peptide and transmembrane domain. The receptor for
CRIPTO-1 has not been identified, yet it has been shown to function as a growth
10   factor. The gene can act as a dominantly transforming oncogene *in vitro*. A
number of studies have shown elevated expression of CRIPTO-1 mRNA and
protein in several human cancers, including human primary breast, gastric, colon,
pancreatic, and bladder (84-88). It is therefore an attractive candidate tumor
associated antigen for specific, active immunotherapy. Peptides have been
15   synthesized with HLA-A2.01 binding potential based on the published sequences
of CRIPTO-1, and these peptides have been used for immunization of HhD
transgenic mice (see Table 9). Figures 24a-c show that some of the tested
peptides (C1, C8, C10, and C12) can elicit lymphocyte response which
specifically lyses CRIPTO-1 peptide-loaded target cells.

20

### TABLE 9
### Predicted human Cripto-1 derived peptides that bind to HLA-A2
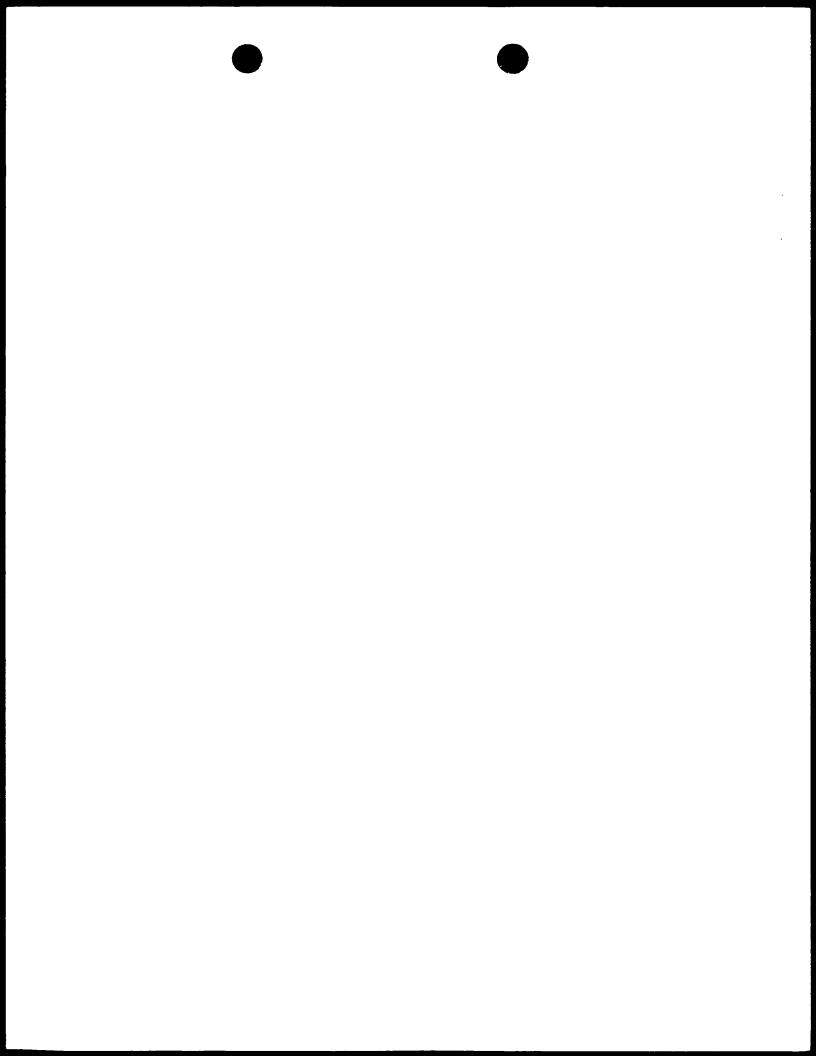
| Peptide | Start Position | Sequence |
|---|---|---|
| Cripto-1/C1 | 5 | KMARFSYSV |
| Cripto-1/C2 | 151 | GLVMDEHLV |
| Cripto-1/C3 | 145 | FLPGCDGLV |
| Cripto-1/C4 | 89 | CMLGSFCAC |
| Cripto-1/C5 | 43 | YLAFRDDSI |
| Cripto-1/C6 | 123 | WLPKKCSLC |
| Cripto-1/C7 | 83 | CLNGGTCML |
| Cripto-1/C8 | 176 | MLVGICLSI |
| Cripto-1/C9 | 23 | FELGLVAGL |
| Cripto-1/C10 | 5 | KMVRFSYSV |
| Cripto-1/C11 | 83 | CLNEGTCML |
| Cripto-1/C12 | 176 | MLAGICLSI |

25   Table 9 shows the sequences of the peptides tested in single letter amino acid
code and their starting position in the intact protein (according to NCBI
accession nos. 117473 (C1-C9) and 321120 (C10-C12).

Although the invention has been described in conjunction with specific
30   embodiments thereof, it is evident that many alternatives, modifications and
variations will be apparent to those skilled in the art. Accordingly, it is intended
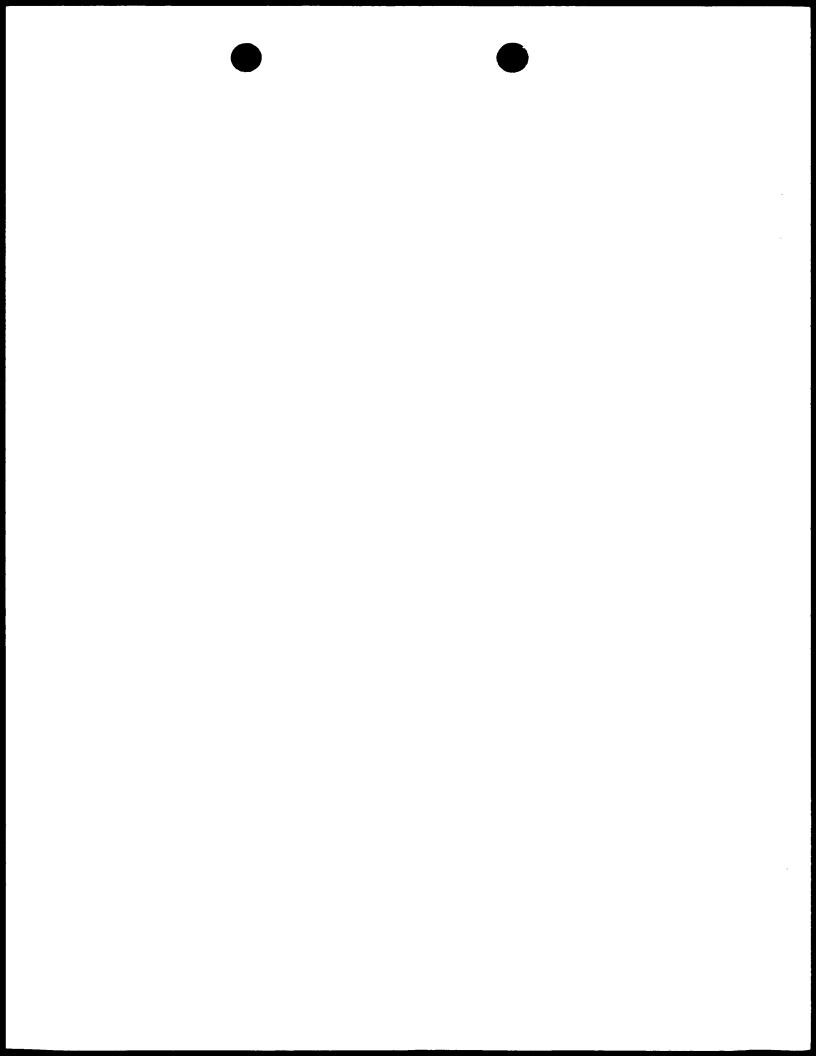
to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

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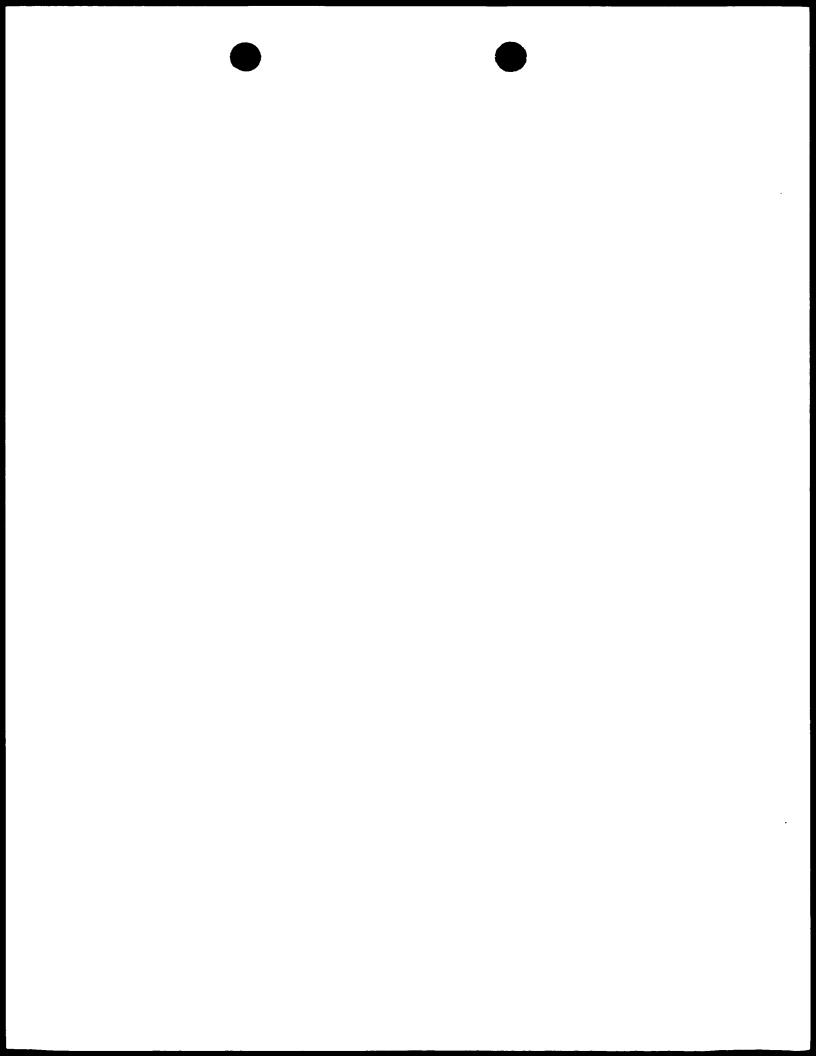
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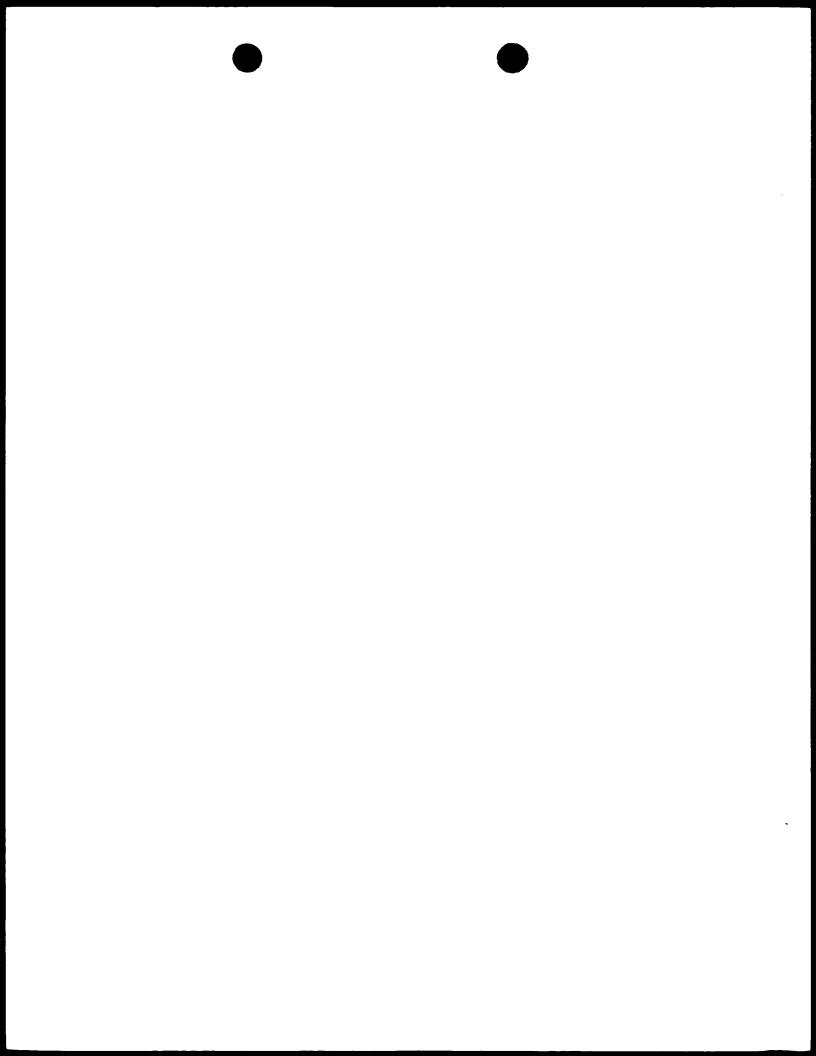
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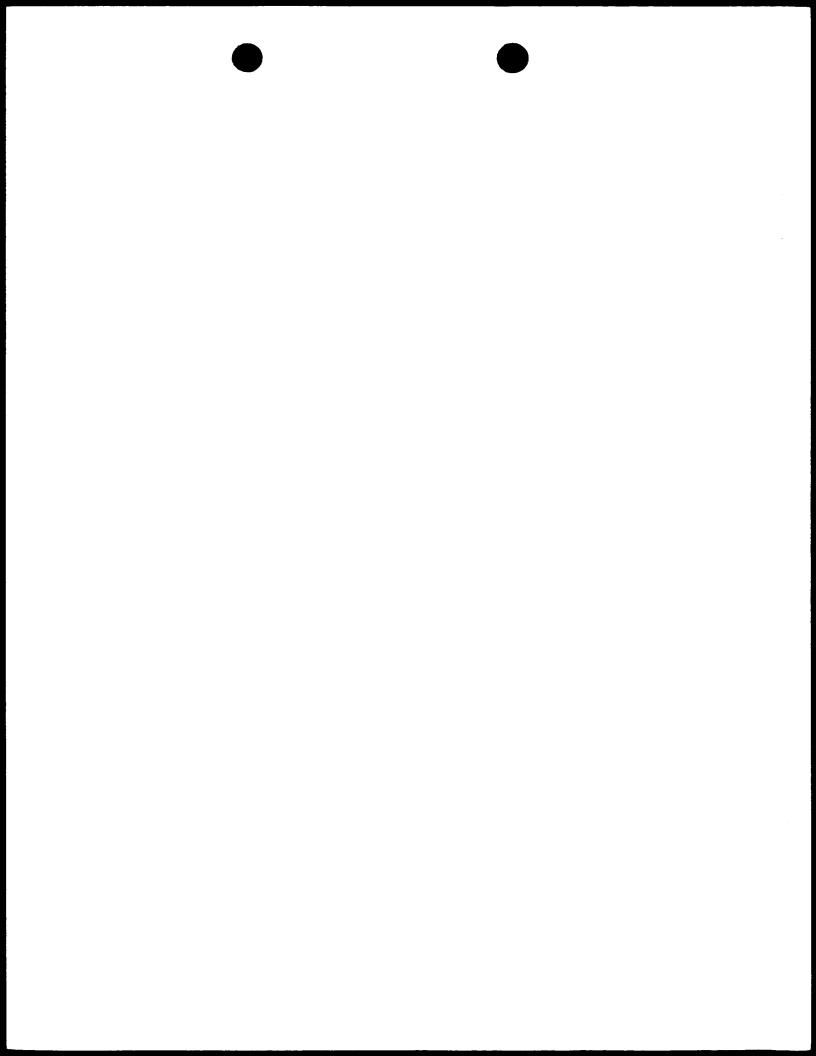
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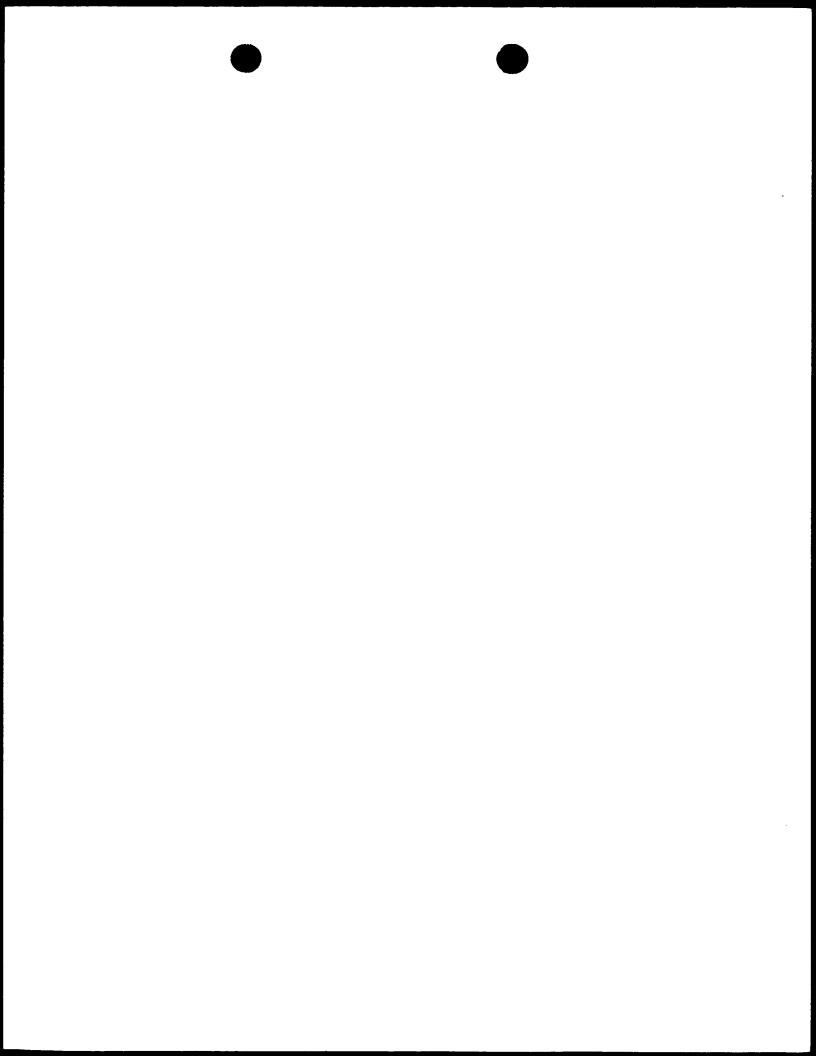
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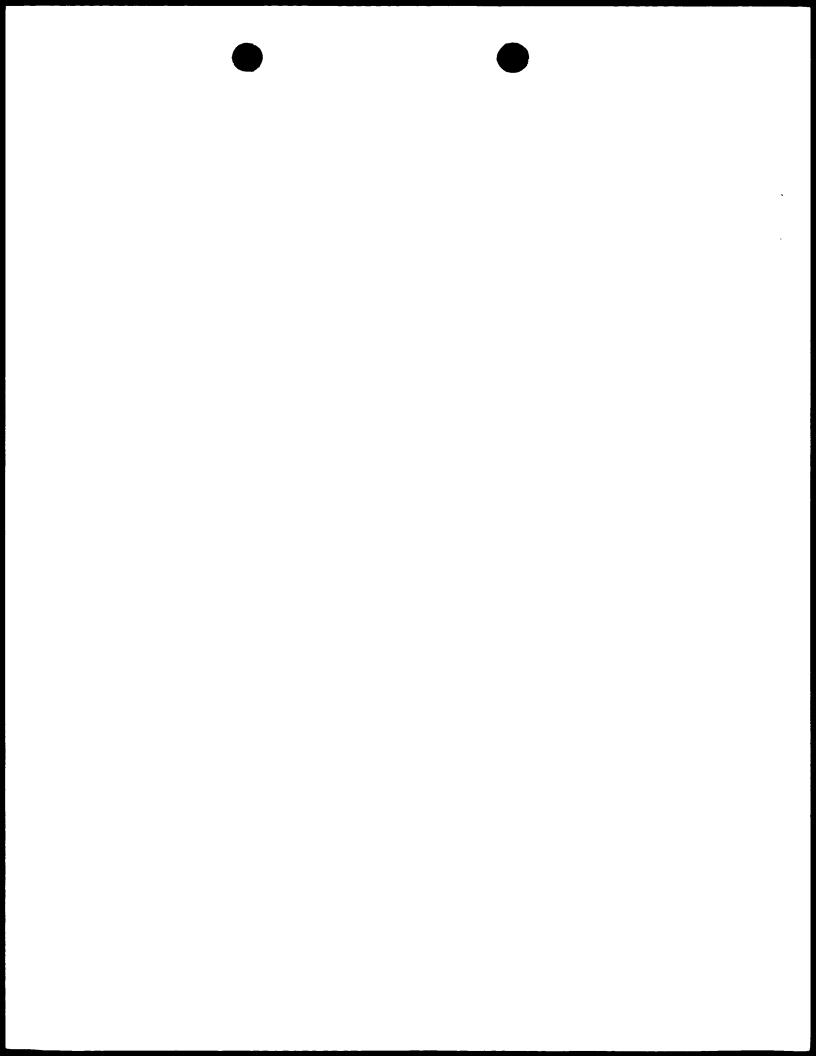
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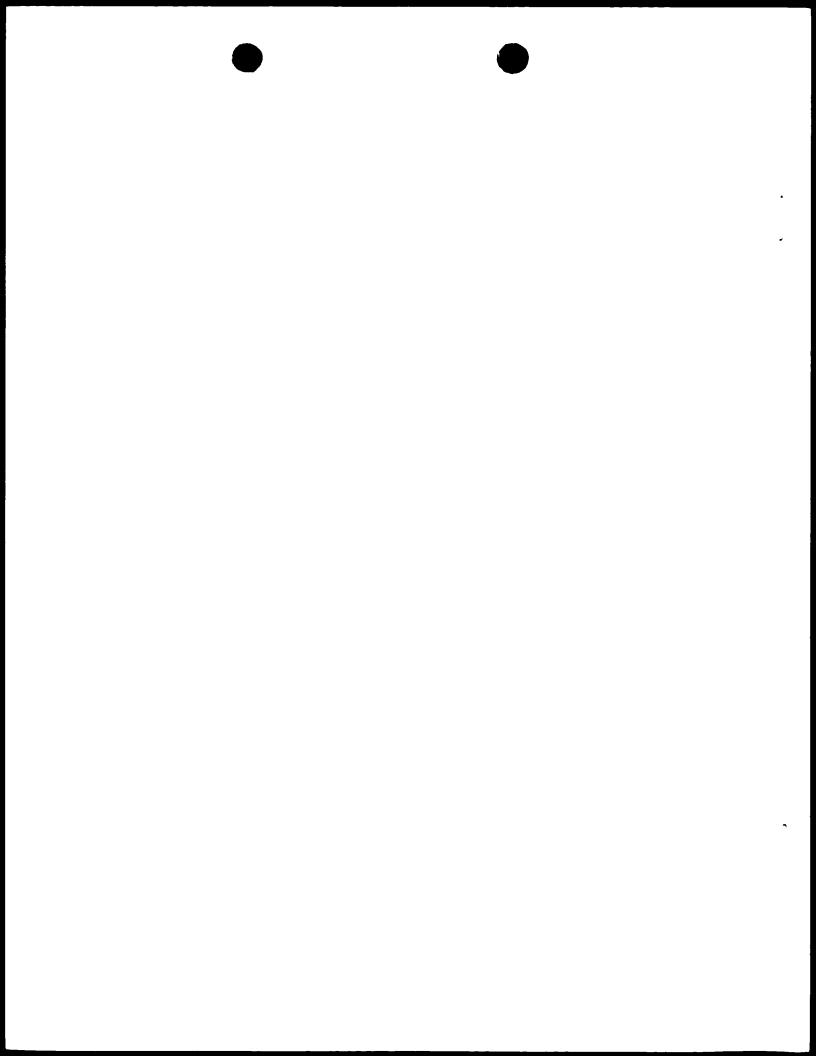
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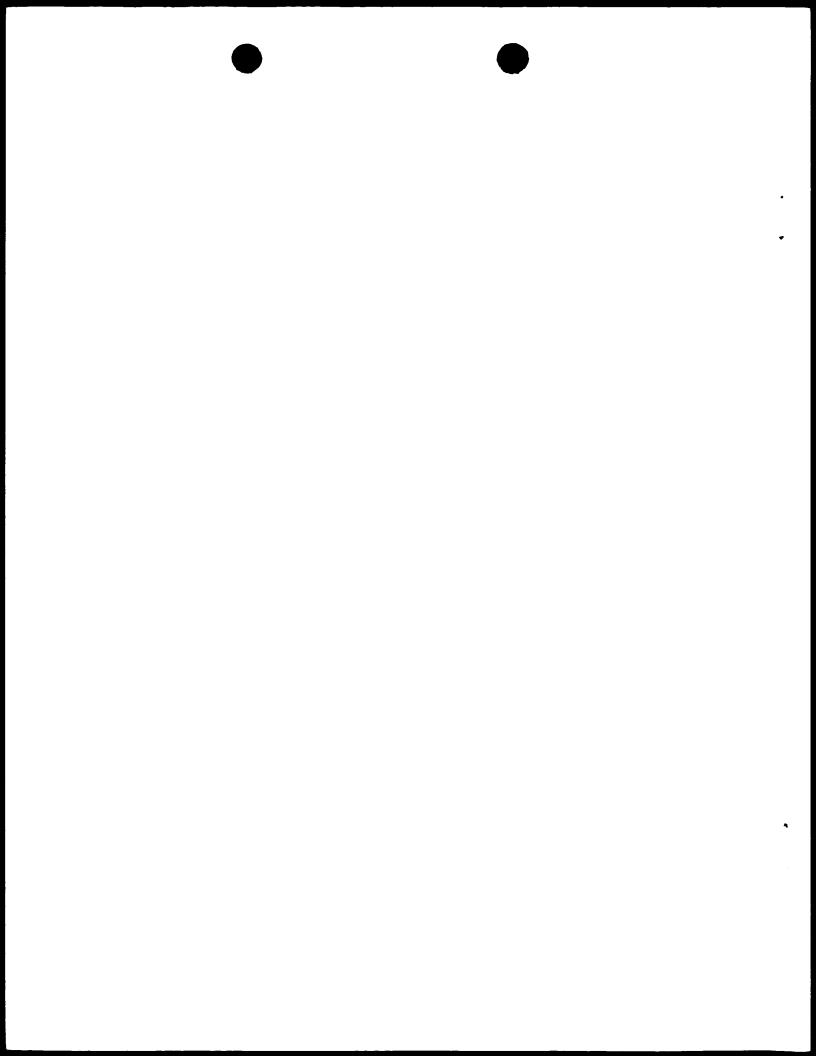
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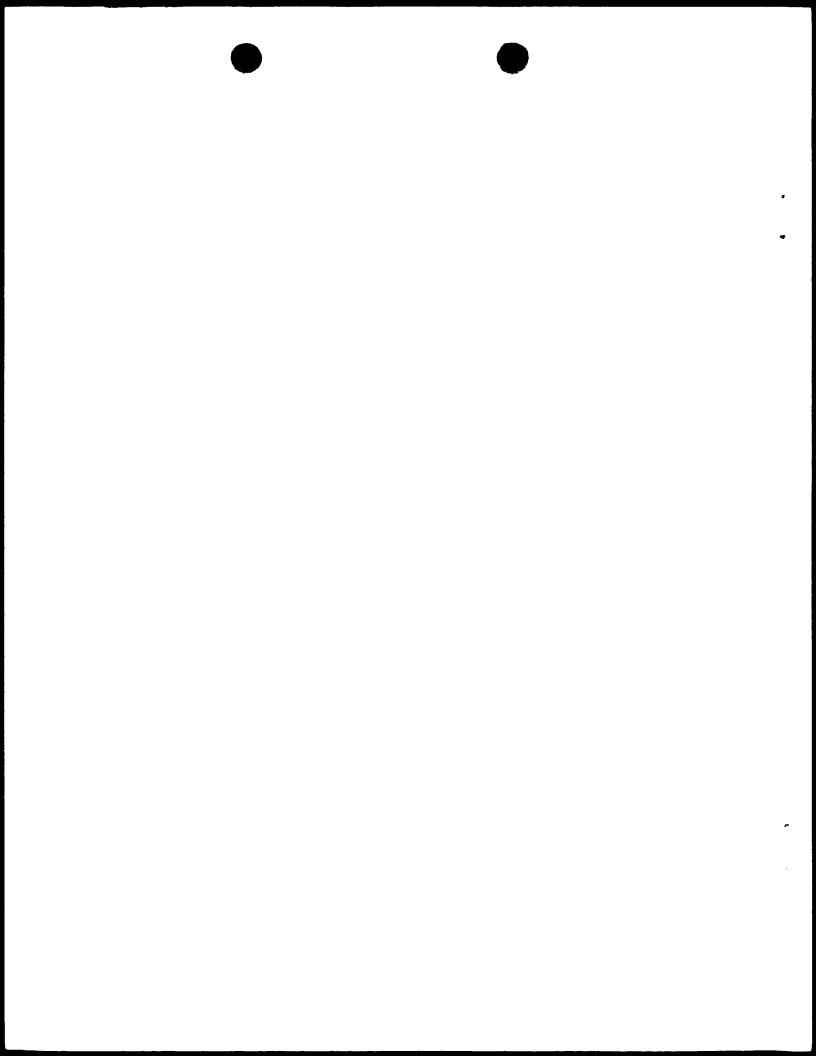
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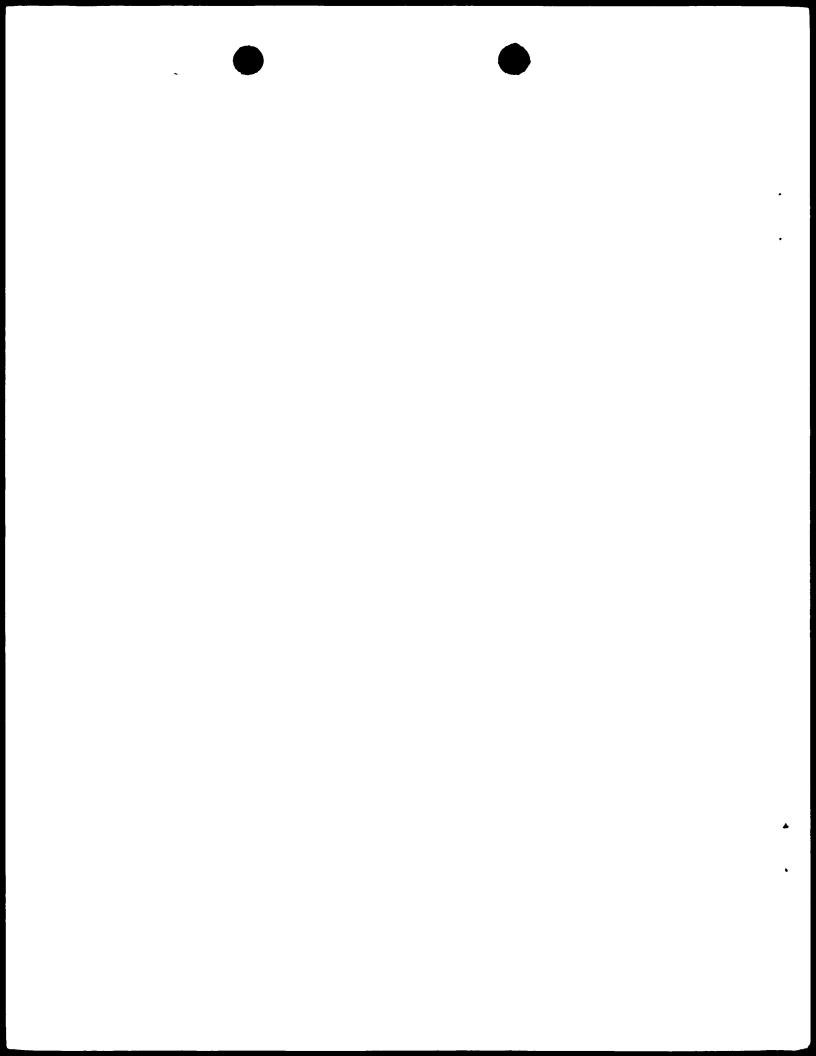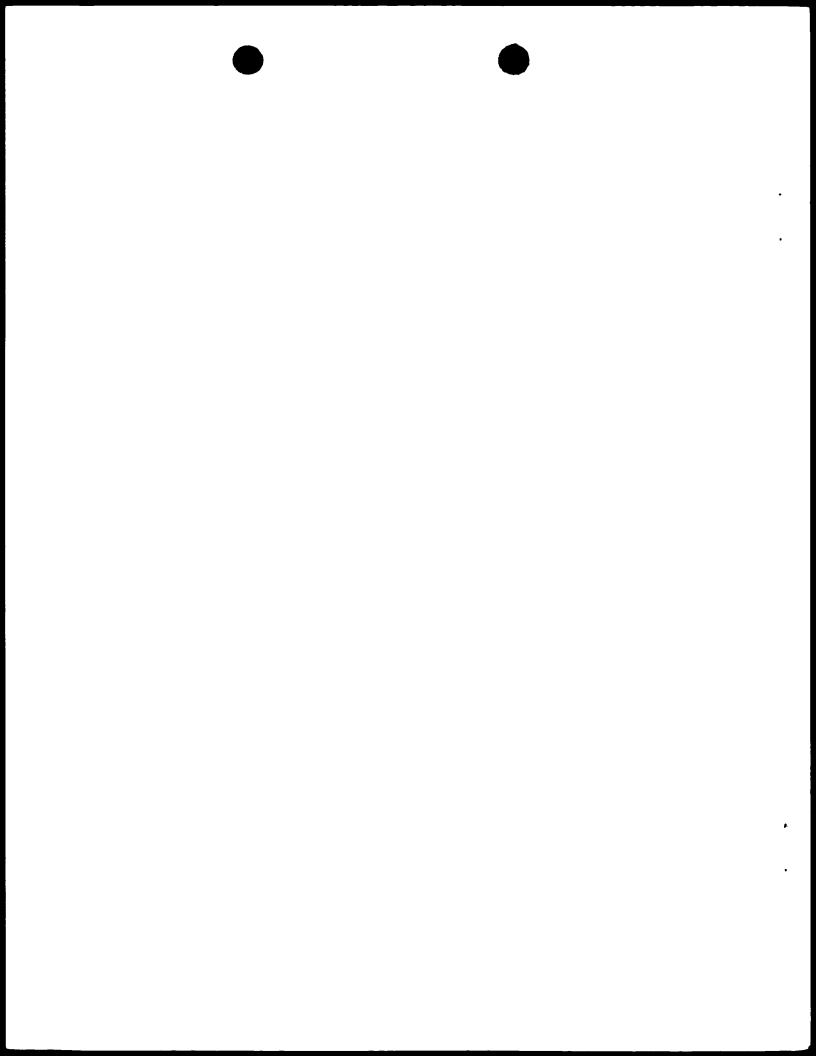
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WHAT IS CLAIMED IS


1.      A peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46), Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8 to 10 amino acid residues, of which a second residue from an amino terminal of the peptide and an end residue at a carboxy terminal of the peptide are hydrophobic or hydrophilic natural or non-natural amino acid residues.


2.      The peptide of claim 1, wherein the peptide is derived from Uroplakin.


3.      The peptide of claim 2, wherein said Uroplakin is selected from the group consisting of Uroplakin II, Uroplakin Ia, Uroplakin III and Uroplakin Ib.


4.      The peptide of claim 3, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:1-19 and 50-64.


5.      The peptide of claim 1, wherein the peptide is derived from said Prostate specific antigen (PSA).


6.      The peptide of claim 5, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:20-24.


7.      The peptide of claim 1, wherein the peptide is derived from said Prostate specific membrane antigen (PSMA).


8.      The peptide of claim 7, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:25-30.


9.      The peptide of claim 1, wherein the peptide is derived from said Prostate acid phosphatase (PAP).


10.     The peptide of claim 9, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:31-34.
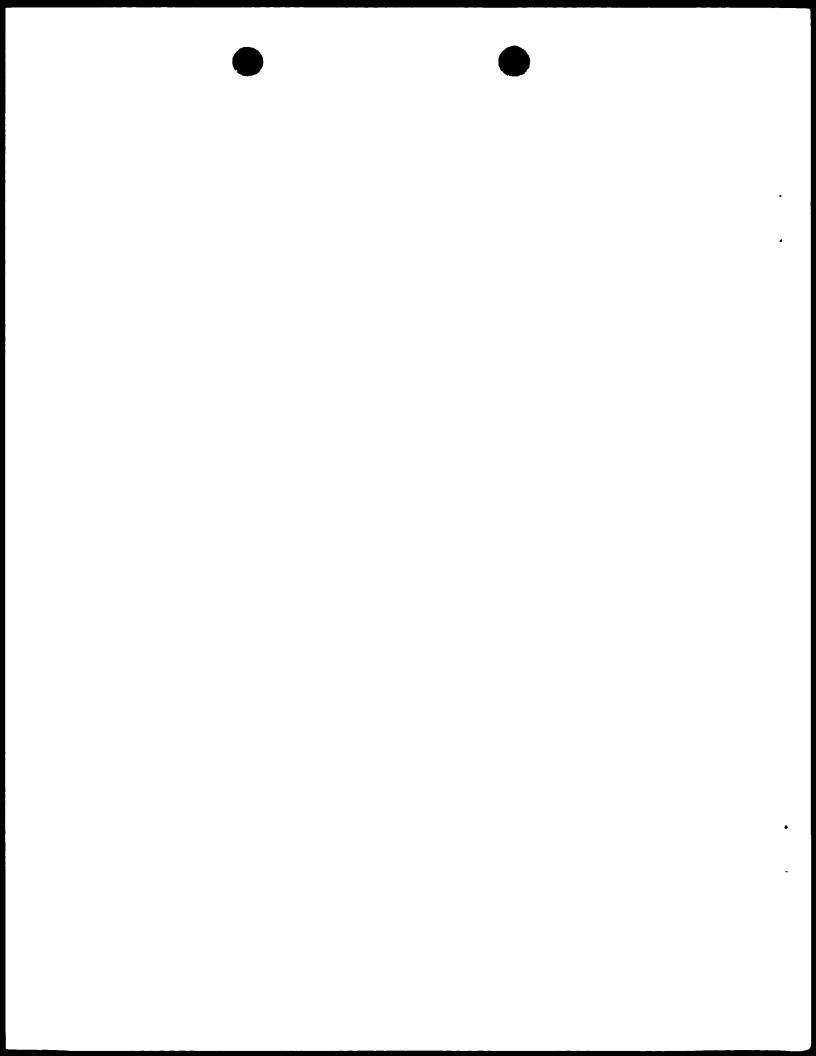
11.    The peptide of claim 1, wherein the peptide is derived from said Mucin.

12.    The peptide of claim 11, wherein the peptide is derived from a non-tandem repeat array of said Mucin.

13.    The peptide of claim 11, wherein the peptide is derived from a region selected from the group consisting of a signal peptide, a cytoplasmic domain and an extracellular domain of said Mucin.

14.    The peptide of claim 13, wherein the peptide is derived from a non tandem repeat array of said Mucin.

15.    The peptide of claim 11, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:42-49.

16.    The peptide of claim 1, wherein the peptide is derived from said Lactadherin (BA-46).

17.    The peptide of claim 16, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:35-41.

18.    The peptide of claim 1, wherein the peptide is derived from said Teratocarcinoma-derived growth factor (CRIPTO-1).

19.    The peptide of claim 18, wherein the peptide has the sequence selected from the group consisting of SEQ ID Nos. 66 to 77.

20.    The peptide of claim 1, wherein said Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase    (PAP),    Lactadherin    (BA-46),    Mucin    (MUC1)    and Teratocarcinoma-derived growth factor (CRIPTO-1) are each independently of mammalian origin.

21.    The peptide of claim 20, wherein said mammal is selected from the group consisting of a humanoid and a rodent.
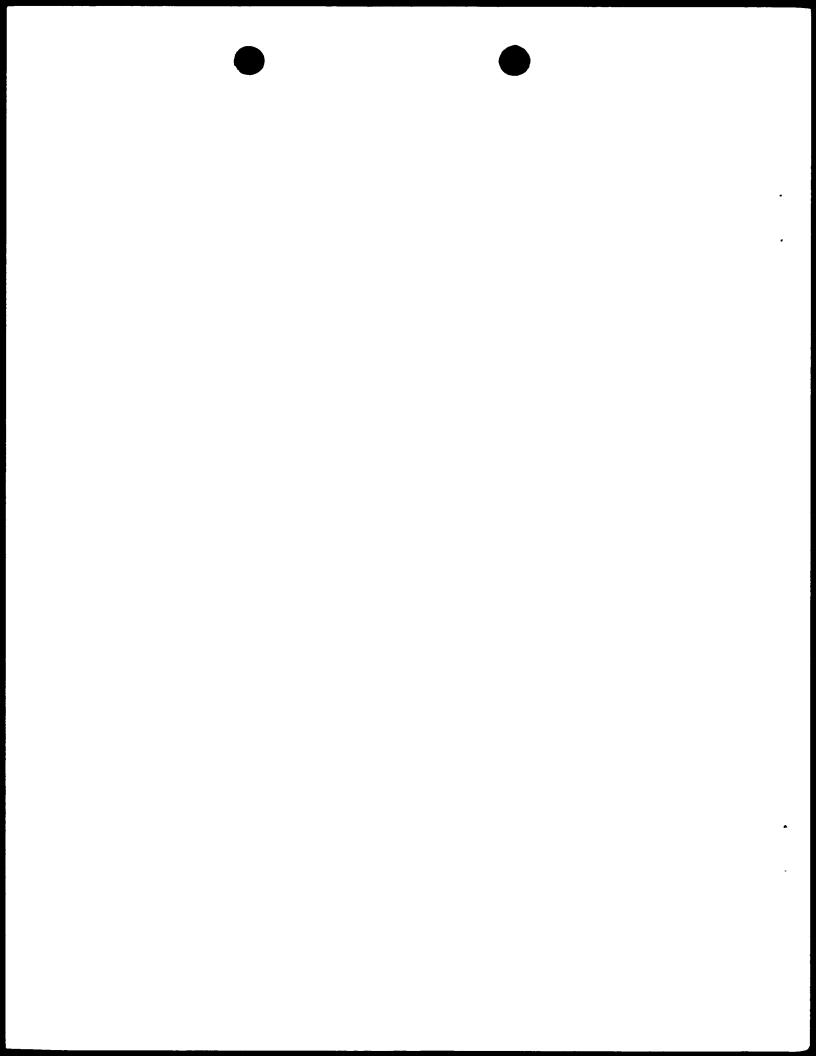
22. The peptide of claim 1 having a sequence selected from the group consisting of SEQ ID NOs:1-64 and 66 to 77.

23. The peptide of claim 1, wherein said peptide includes at least one non-natural modification.

24. The peptide of claim 23, wherein said non-natural modification renders peptides more immunogenic or more stable.

25. The peptide of claim 23 or 24, wherein said at least one modification is selected from the group consisting of peptoid modification, semipeptoid modification, cyclic peptide modification, N terminus modification, C terminus modification, peptide bond modification, backbone modification and residue modification.

26. A pharmaceutical composition comprising, as an active ingredient, at least one peptide as set forth in any of claims 1-25 and a pharmaceutically acceptable carrier.

27. The pharmaceutical composition of claim 26, wherein said carrier is selected from the group consisting of a proteinaceous carrier to which said at least one tumor associated antigen peptide is linked, an adjuvant, a protein or a recombinant protein and an antigen presenting cell.

28. The pharmaceutical composition of claim 26, wherein the composition is effective in prevention or cure of cancer or cancer metastases.

29. The pharmaceutical composition of claim 28, wherein said cancer is selected from the group consisting of breast, bladder, prostate, pancreas, ovary, thyroid, colon, stomach and head and neck cancer.

30. The pharmaceutical composition of claim 28, wherein said cancer is a carcinoma.

31. The pharmaceutical composition of claim 26, wherein the composition is a vaccine.
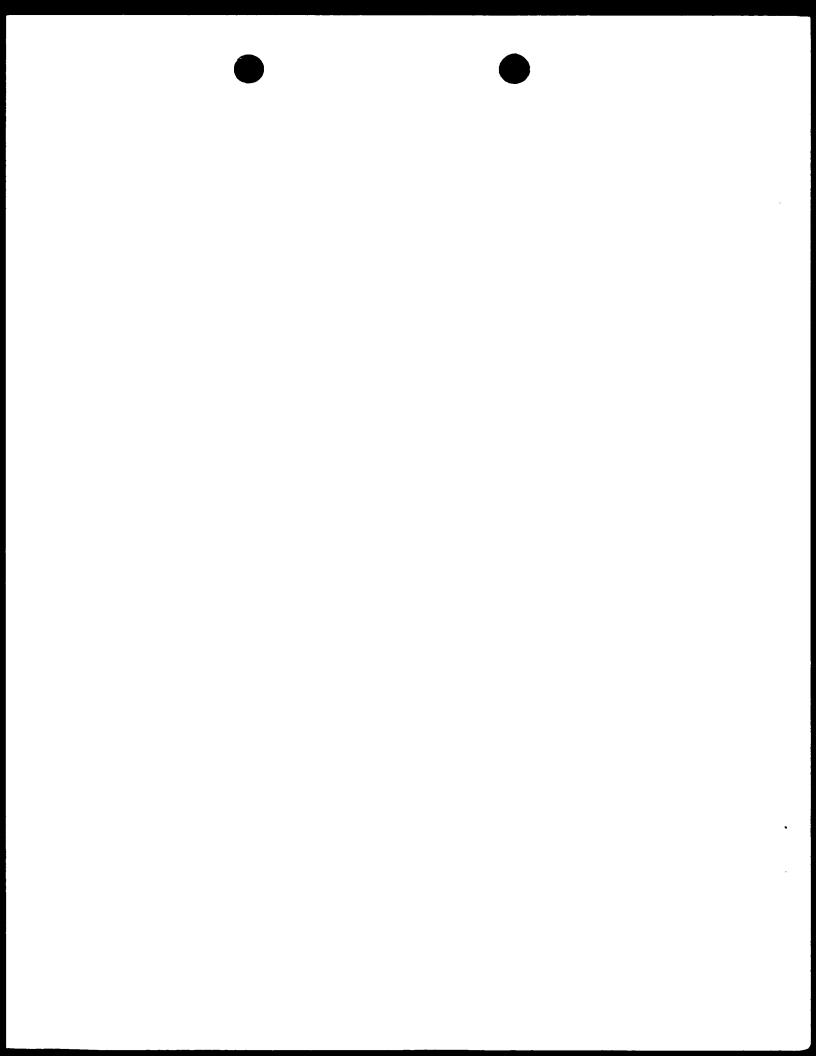
32.     A vaccine composition comprising, as an active ingredient, at least one peptide as set forth in any of claims 1-25 and a suitable carrier.

33.     The vaccine composition of claim 32, wherein said carrier is selected from the group consisting of a proteinaceous carrier to which said at least one tumor associated antigen peptide is linked, an adjuvant, a protein or a recombinant protein and an antigen presenting cell.

34.     The vaccine composition of claim 32, wherein the composition is effective in prevention or cure of cancer or cancer metastases.

35.     The vaccine composition of claim 34, wherein said cancer is selected from the group consisting of breast, bladder, prostate, pancreas, ovary, thyroid, colon, stomach and head and neck cancer.

36.     The vaccine composition of claim 34, wherein said cancer is a carcinoma.

37.     A method of prevention or cure of a cancer or of metastases thereof comprising the step of administering to a patient an effective amount of the pharmaceutical composition of any of claims 26-31.

38.     A method of prevention or cure of a cancer or of metastases thereof comprising the step of vaccinating a patient with an effective amount of the vaccine composition of any of claims 32-36.

39.     A polynucleotide encoding at least one peptide according to any of claims 1-25.

40.     A polynucleotide encoding at least one peptide according to any of the SEQ ID Nos. 1 to 64 and 66 to 77.

41.     The polynucleotide of claim 39 or 40, wherein the polynucleotide forms a part of a longer polynucleotide designed to encode a fused protein product from which said at least one peptide is cleavable by a protease.
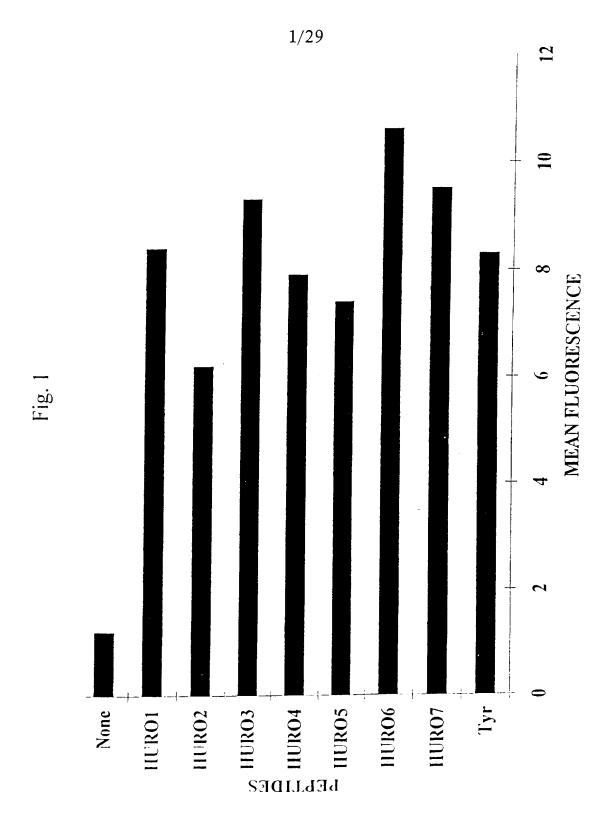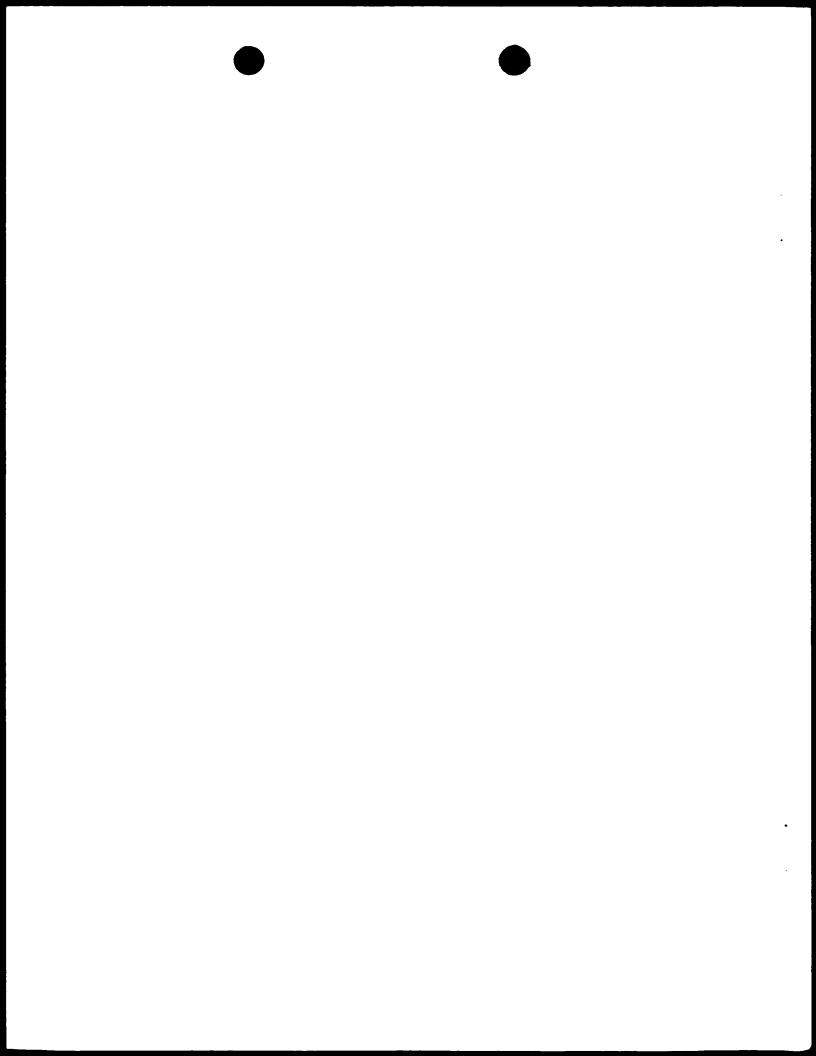
42. A pharmaceutical composition comprising, as an active ingredient, at least one polynucleotide at set forth in any claims 39-41 and a pharmaceutically acceptable carrier.

43. A cellular vaccine composition comprising an antigen presenting cell presenting at least one peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the at least one peptide comprising 8 to 10 amino acid residues, of which a second residue from an amino terminal of the peptide and a carboxy terminal residue of the peptide are hydrophobic or hydrophilic, natural or non-natural amino acid residues.

44. The cellular vaccine composition of claim 43, wherein said antigen presenting cell is selected from the group consisting of a dendritic cell, a macrophage, a B cell and a fibroblast.

45. The cellular vaccine composition of claim 43, wherein said antigen presenting cell is caused to present said at least one tumor associated antigen peptide by a method selected from the group consisting of:
   (a) genetically modifying said antigen presenting cell with at least one polynucleotide encoding said at least one tumor associated antigen peptide such that said peptide or at least one longer polypeptide including said peptide will be expressed;
   (b) loading said antigen presenting cell with at least one polynucleotide encoding said at least one tumor associated antigen peptide;
   (c) loading said antigen presenting cell with said at least one tumor associated antigen peptide; and
   (d) loading said antigen presenting cell with at least one longer polypeptide including said at least one tumor associated antigen peptide

46. The peptide of claim 1, wherein the second residue and the end residue are neutral, hydrophobic and aliphatic.

47. The pharmaceutical composition of any of claims 26 to 31 and 42 also comprising a helper peptide.
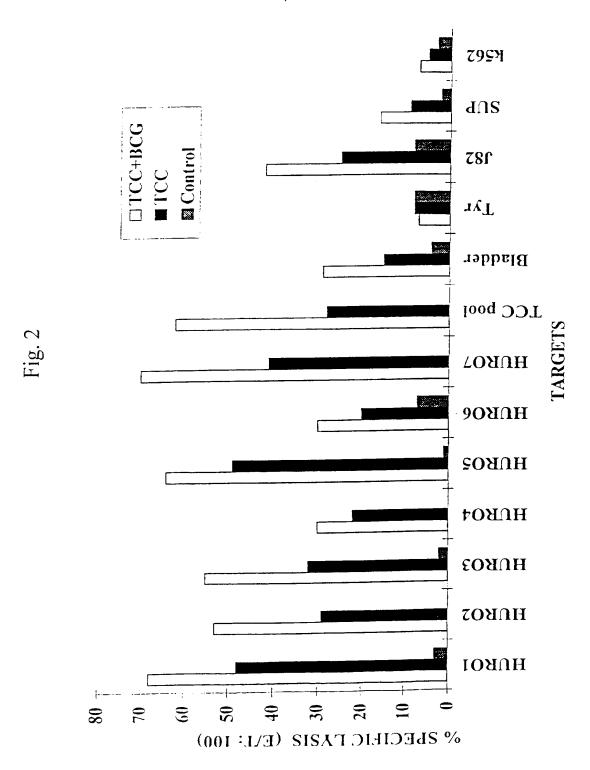
48.    The pharmaceutical composition of claim 47, wherein the helper peptide has a T helper epitope.

49.    The vaccine composition of any of claims 32 to 36 also comprising a helper peptide.

50.    The vaccine composition of claim 49 wherein the helper peptide has a T helper epitope.

51.    Use of the at least one peptide of claims 1 to 25 in the manufacture of a medicament, substantially as described in the specification.

52.    The at least one peptide of claims 1 to 25 for use as a medicament.

53.    Use of the at least one peptide of claims 1 to 25 in the manufacture of a medicament for the prevention or cure of a cancer or cancer metastases, substantially as described in the specification.

54.    The at least one peptide of claims 1 to 25 for use as a medicament for the prevention or cure of a cancer or cancer metastases.

55.    A peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46) and Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8-10 amino acid residues as selected so as to promote effective binding to a MHC class 1 type molecule such that a CTL response is elicitable.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig.18

Fig. 19



Breast T.E. ■
Breast N.E. □

% Specific lysis (E:T 50:1)

Effectors: MUC-1/A7, MUC-1/E6, MUC-1/D6

Fig. 20

Fig. 21



Fig. 22

Fig. 23a

Fig. 23b

Fig. 23c

Fig. 23d

Fig. 23e

Fig. 24a

Fig. 24b

Fig. 24c

SEQUENCE LISTING

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     Bio-Technology General Corp.

<120> Tumor Associated Antigen Peptides and Use of Same as
     Anti-Tumor Vaccines

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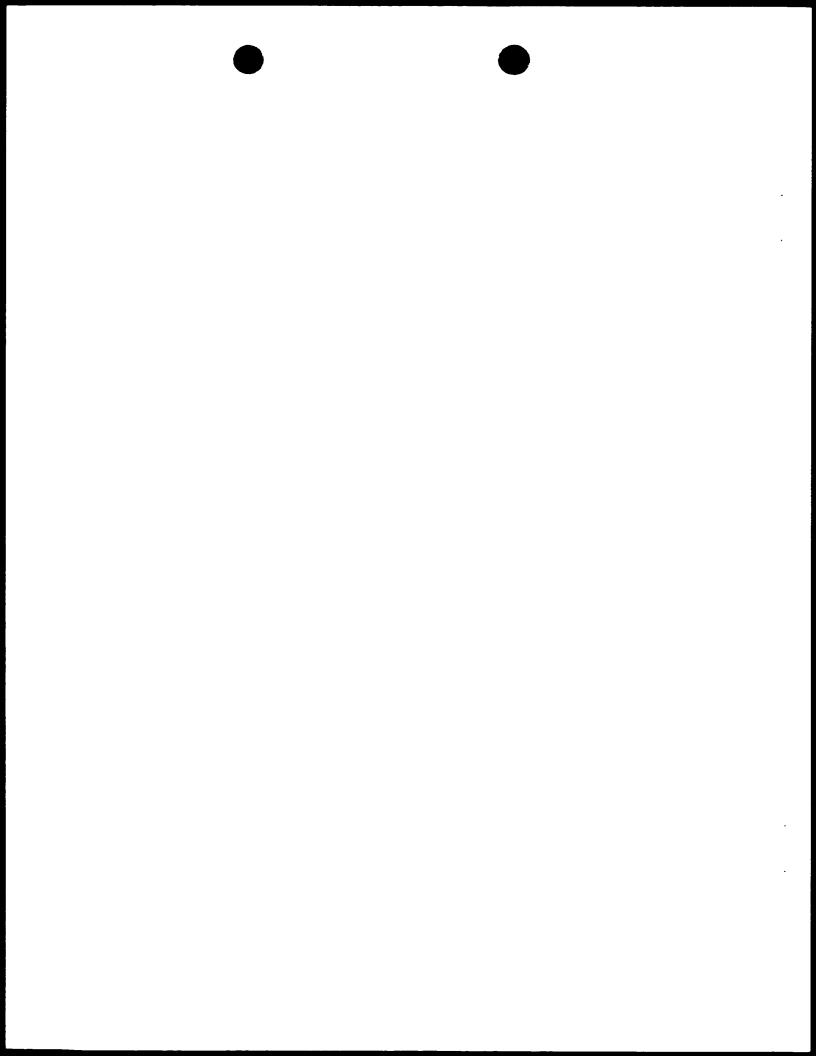
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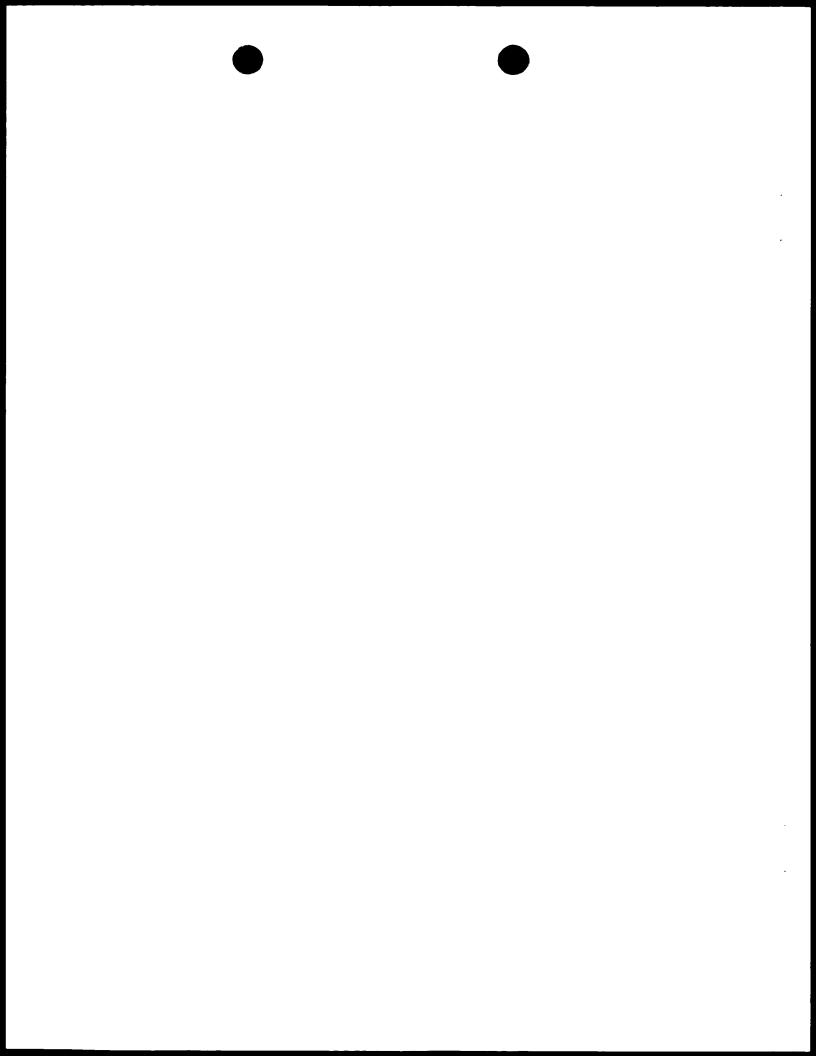
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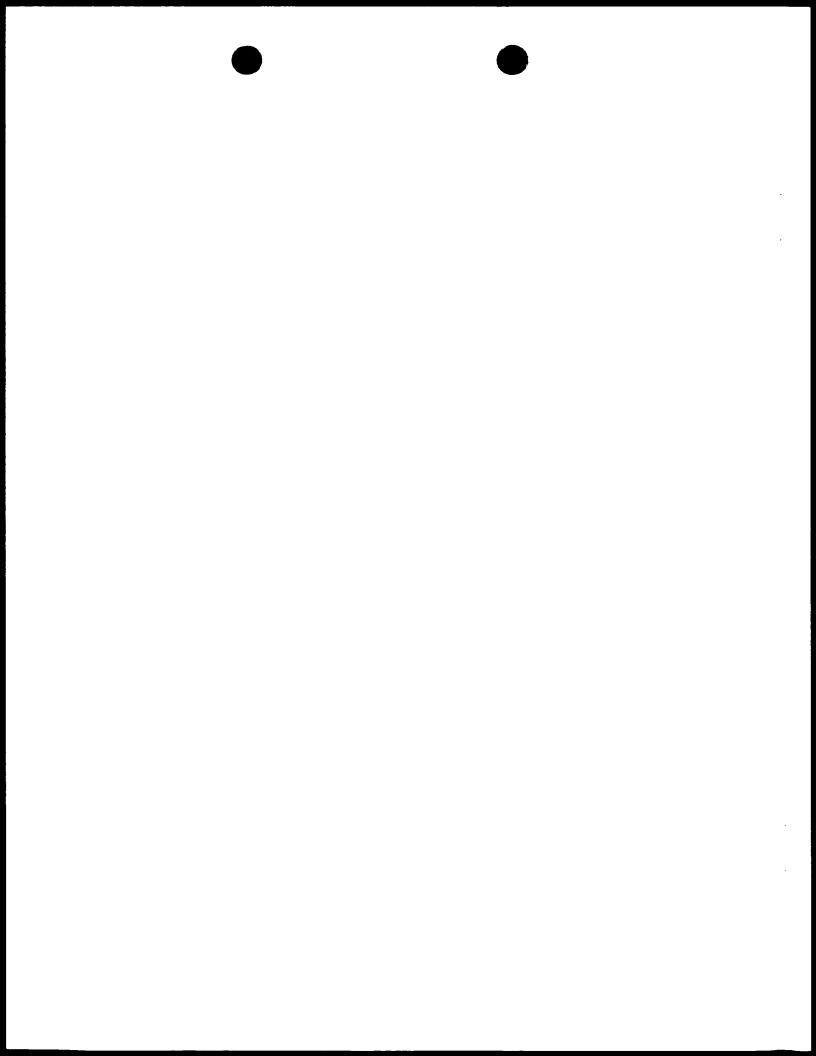
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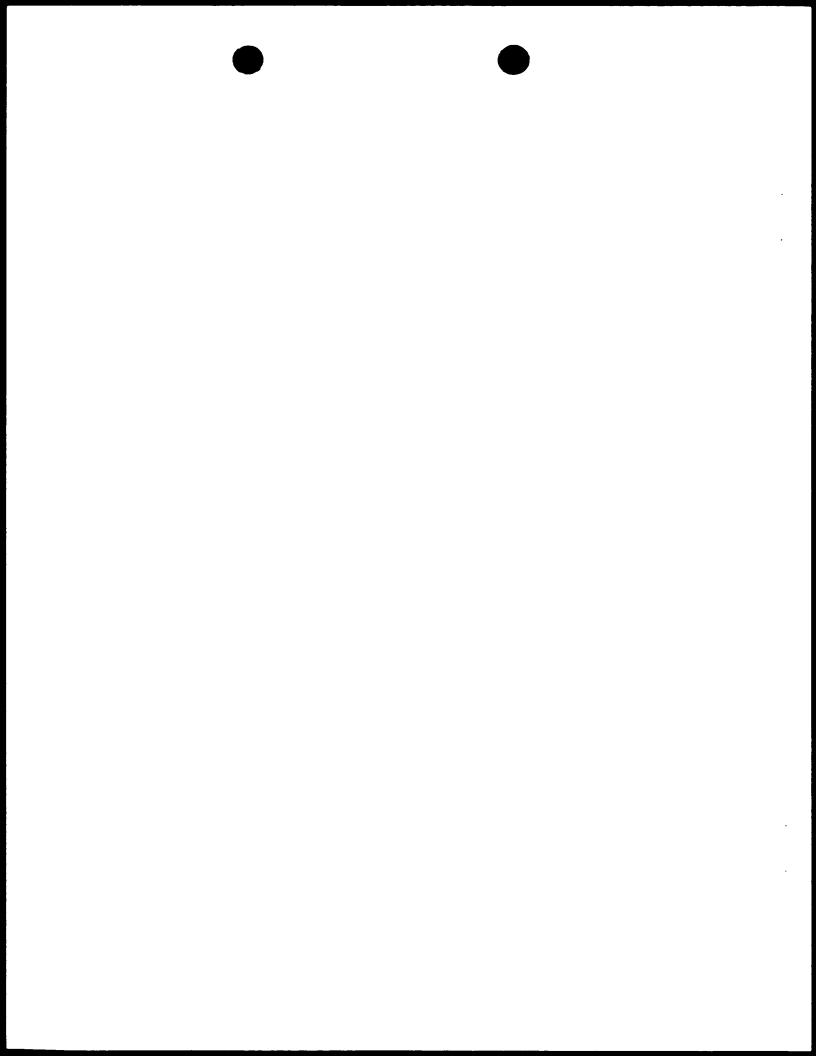
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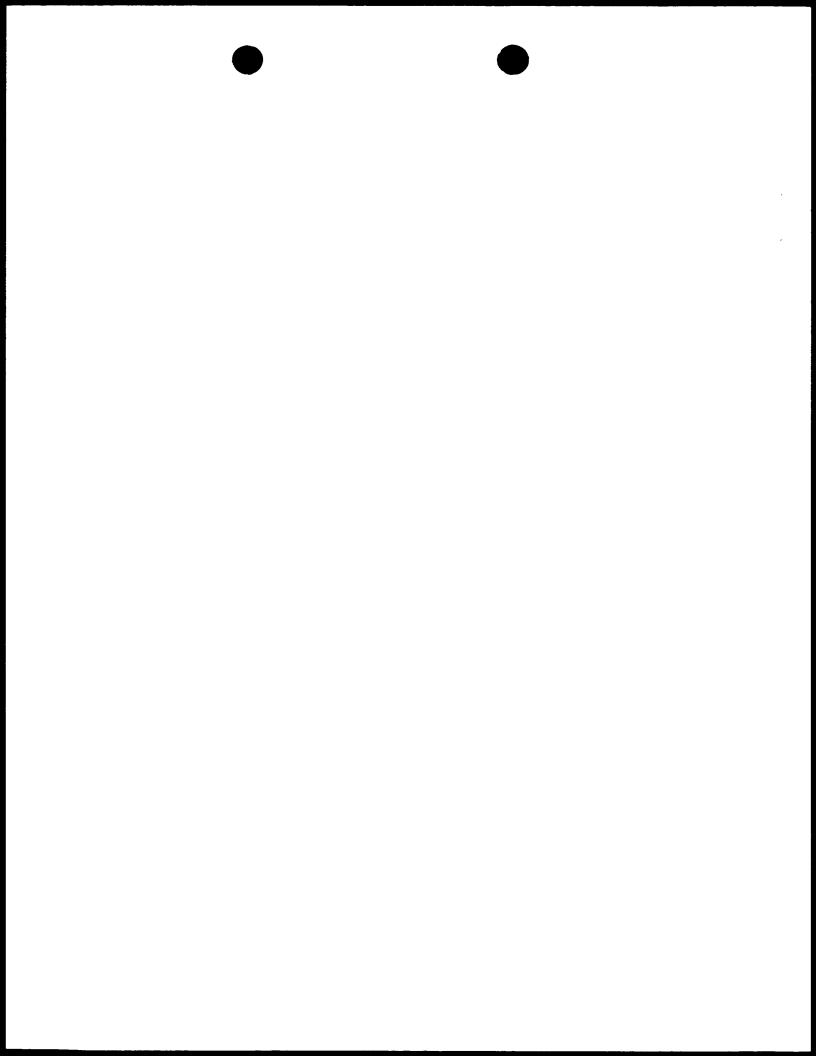
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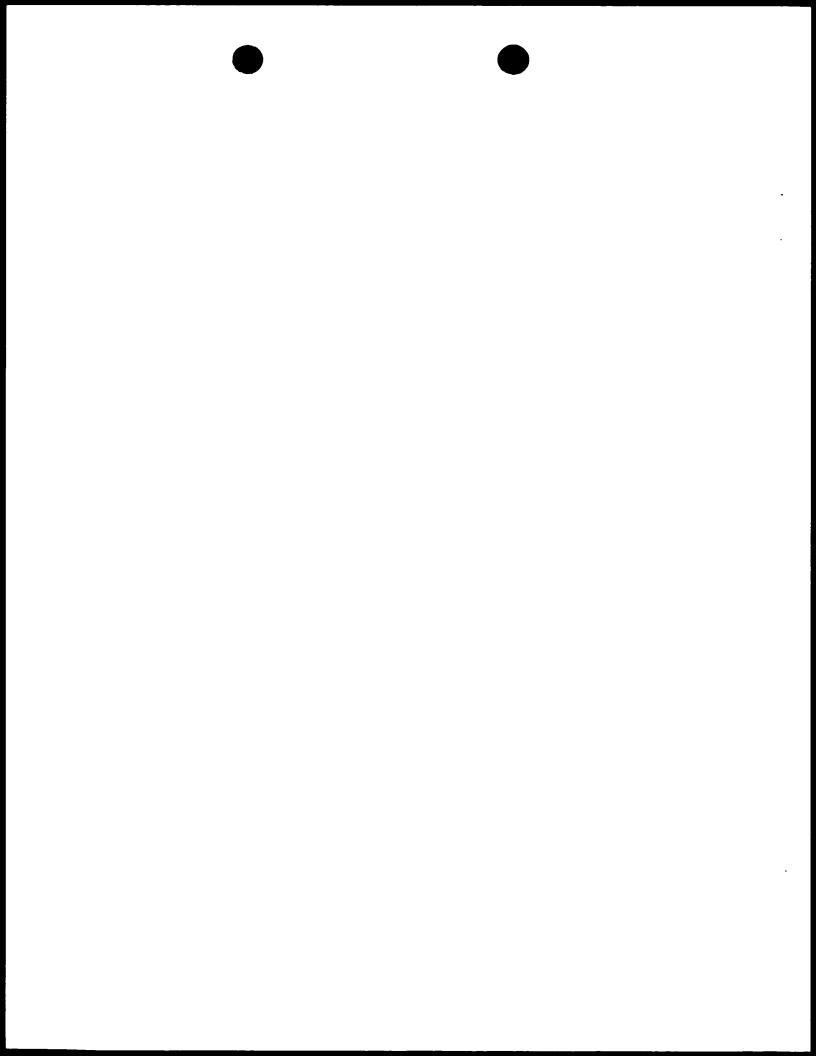
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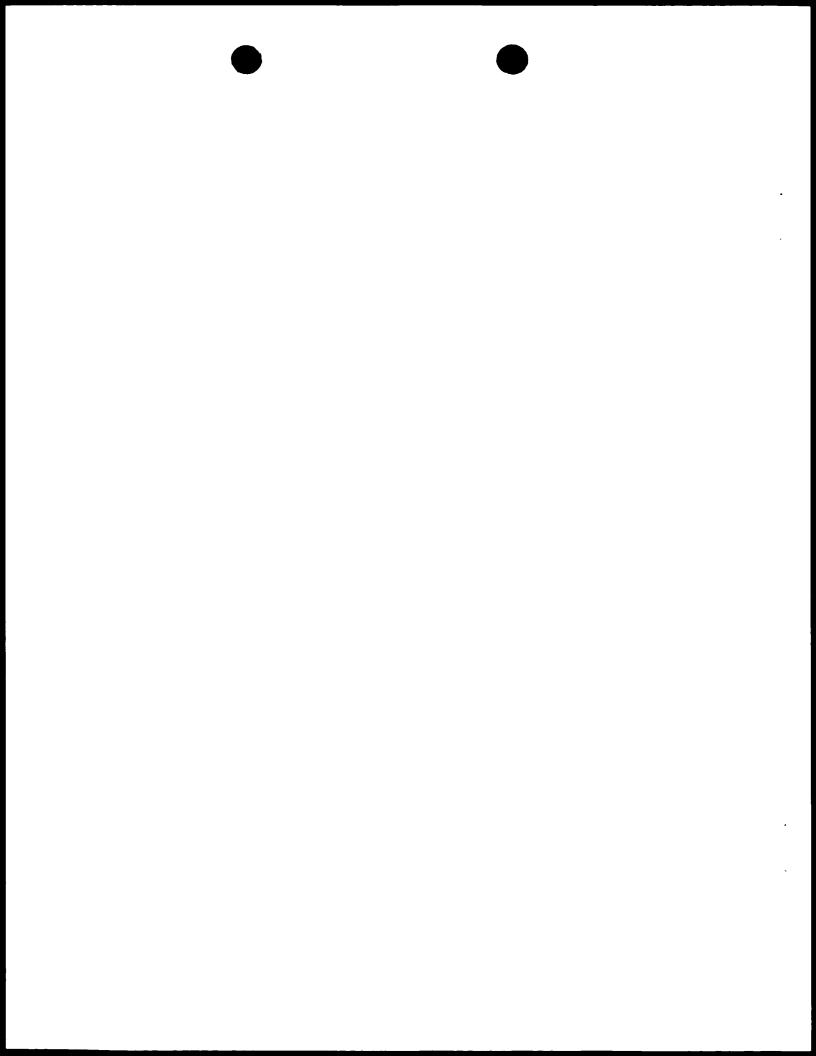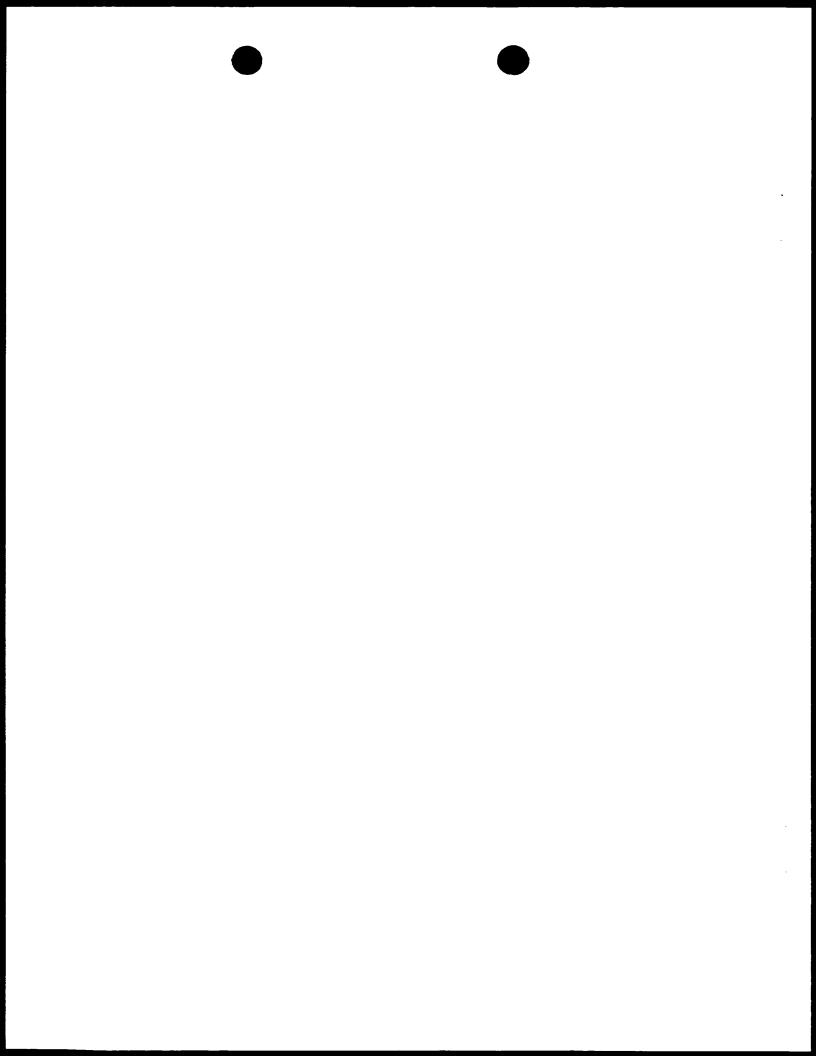
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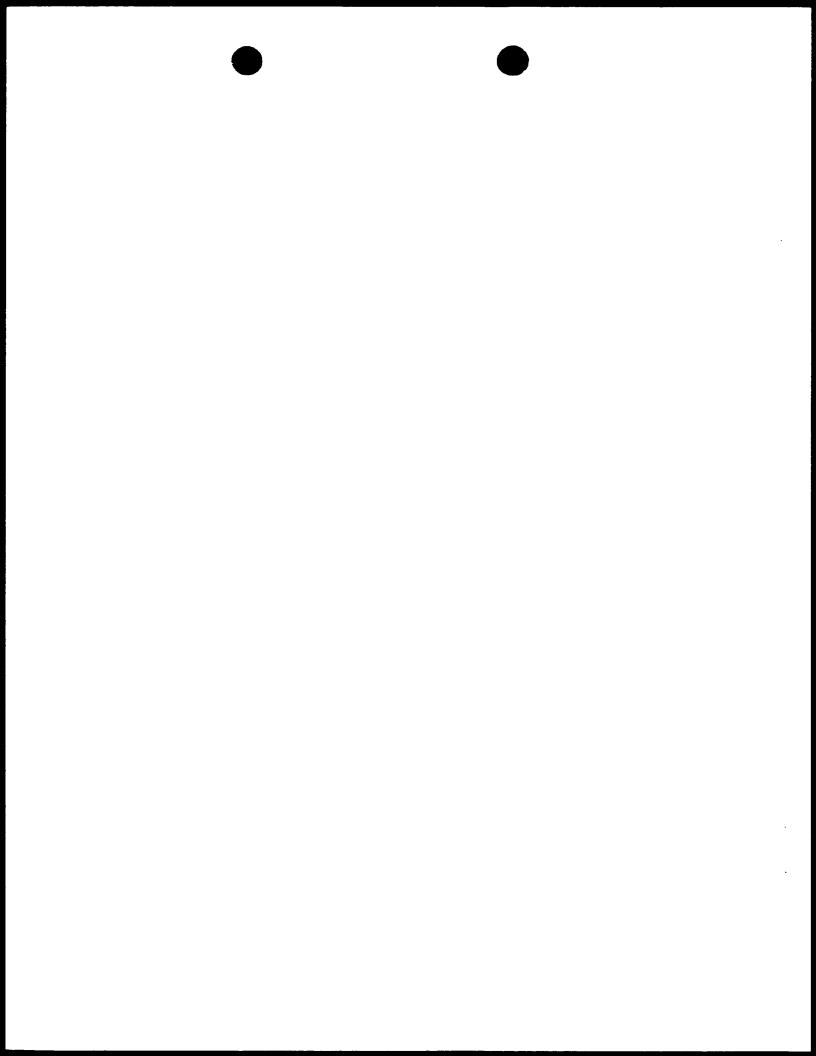

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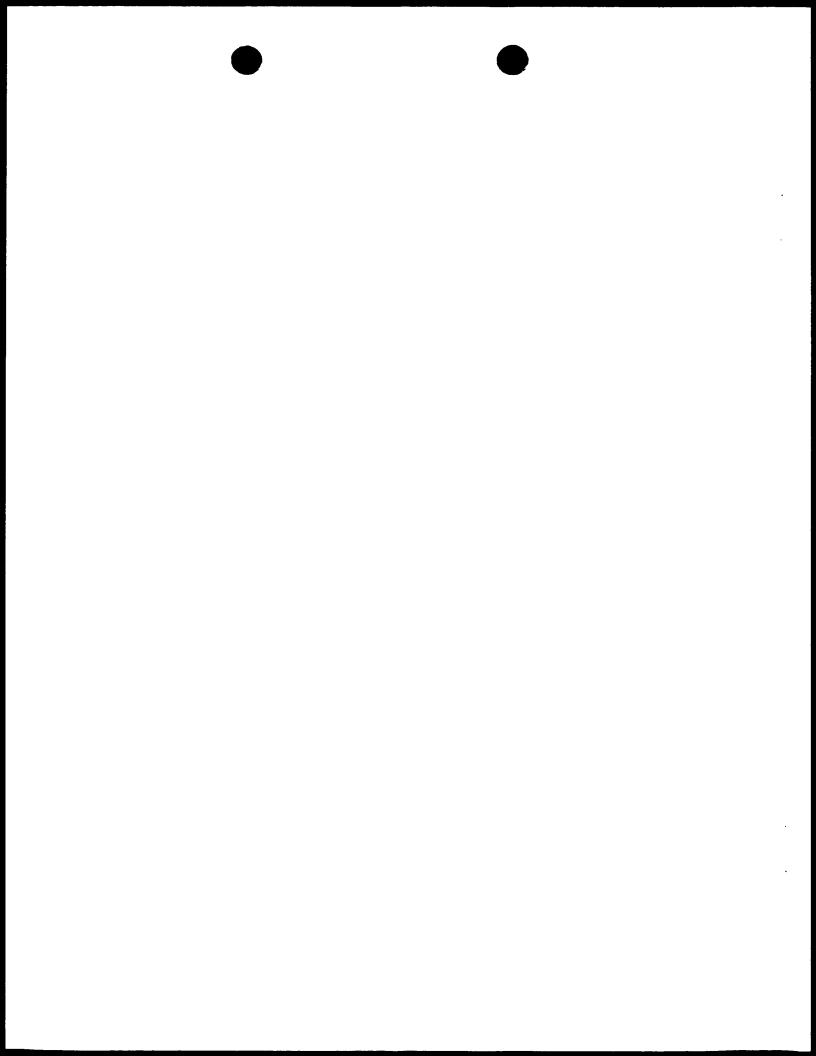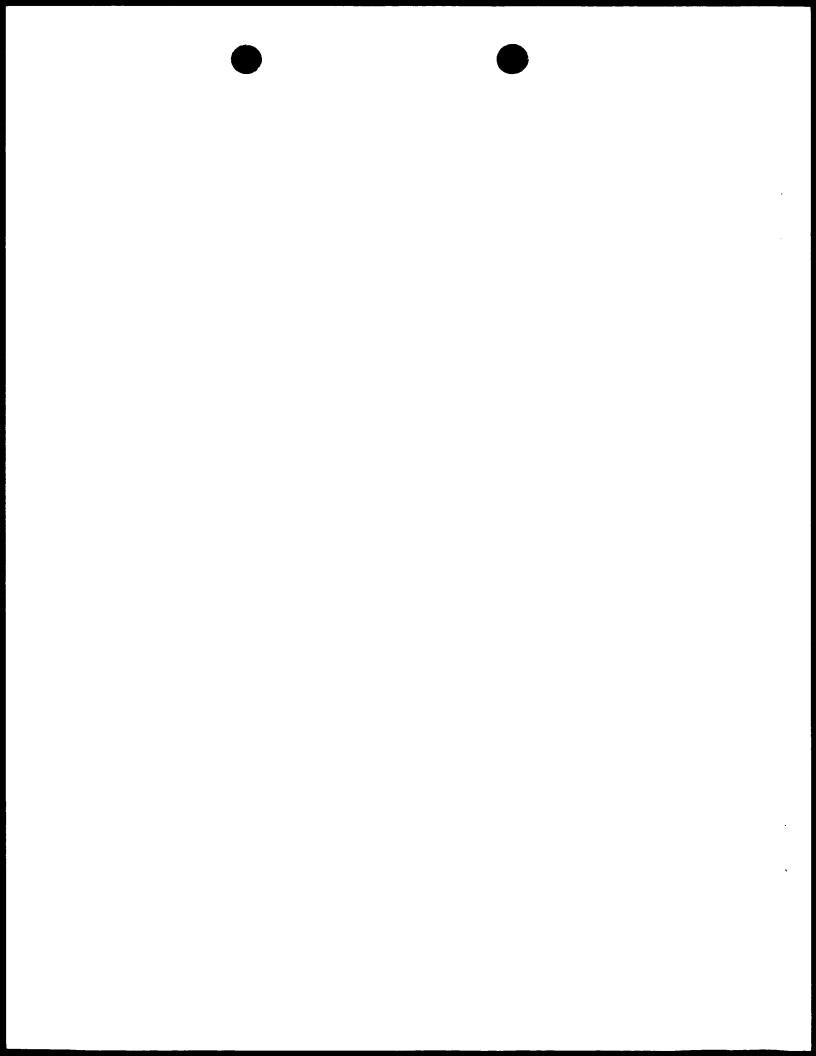
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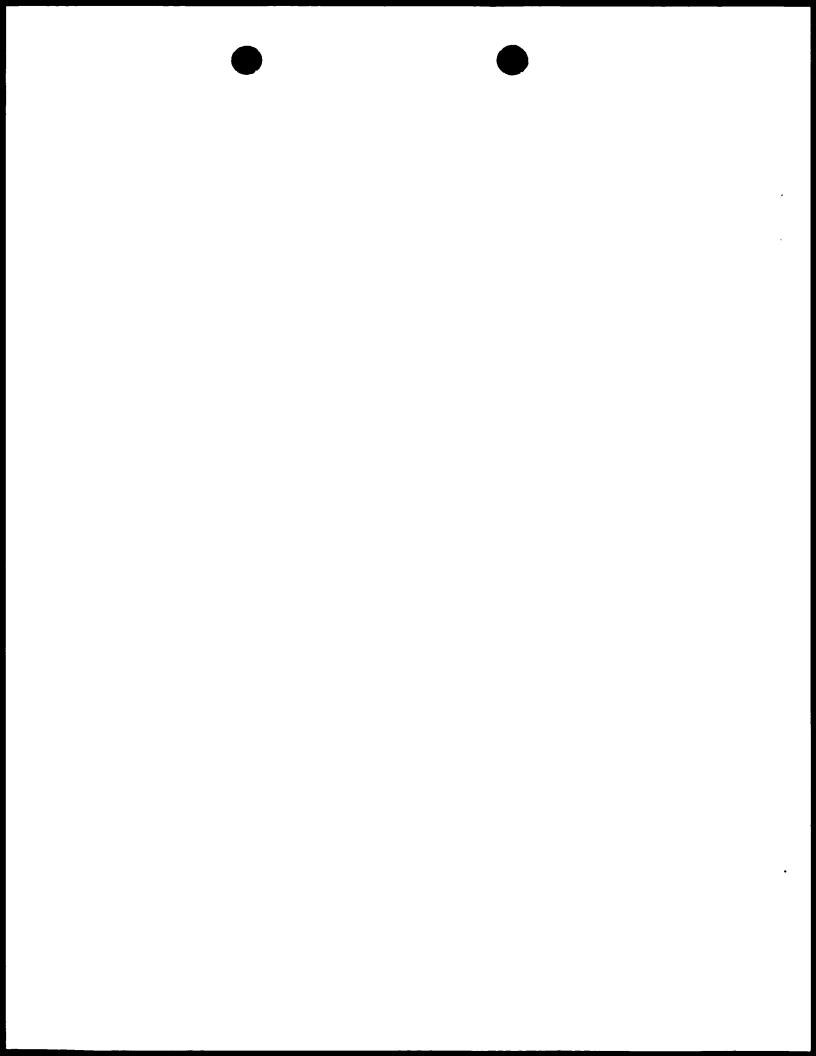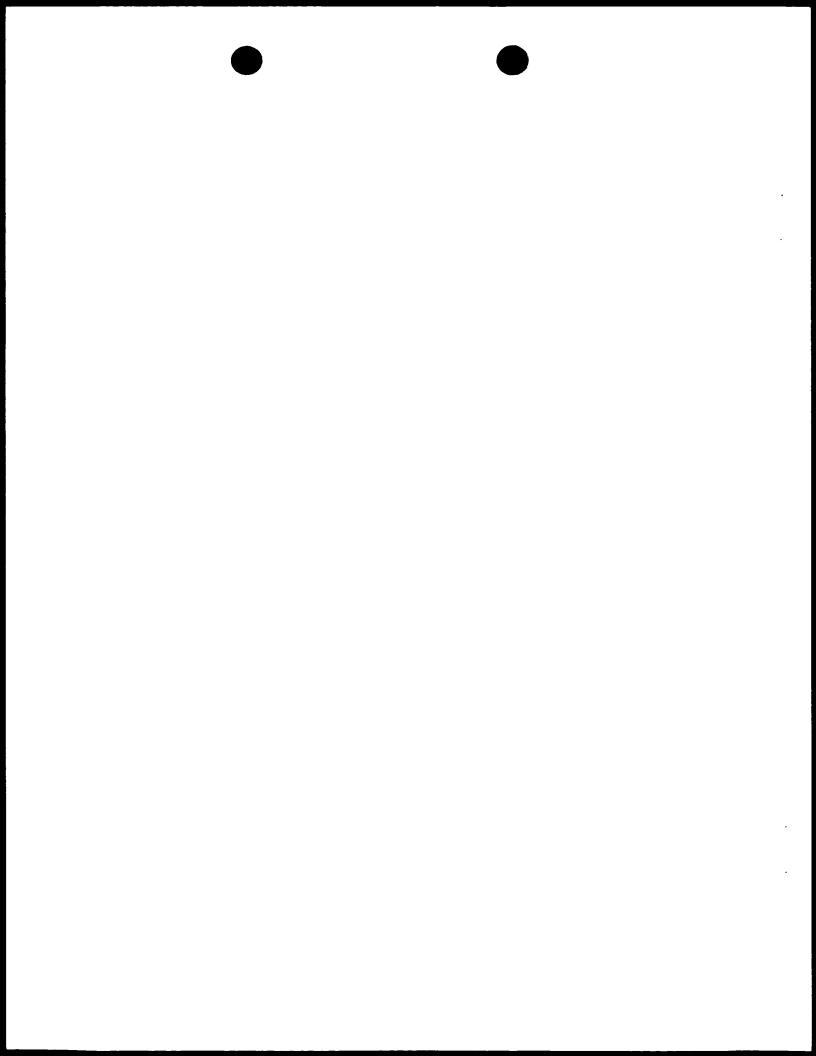
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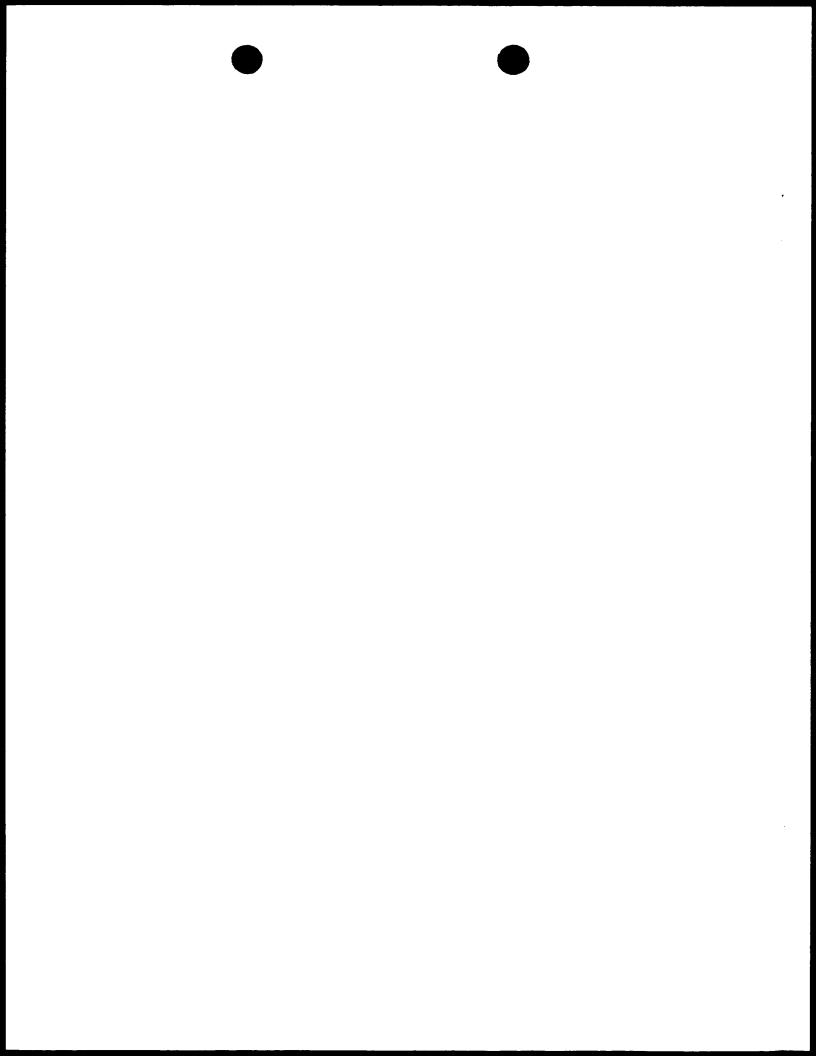
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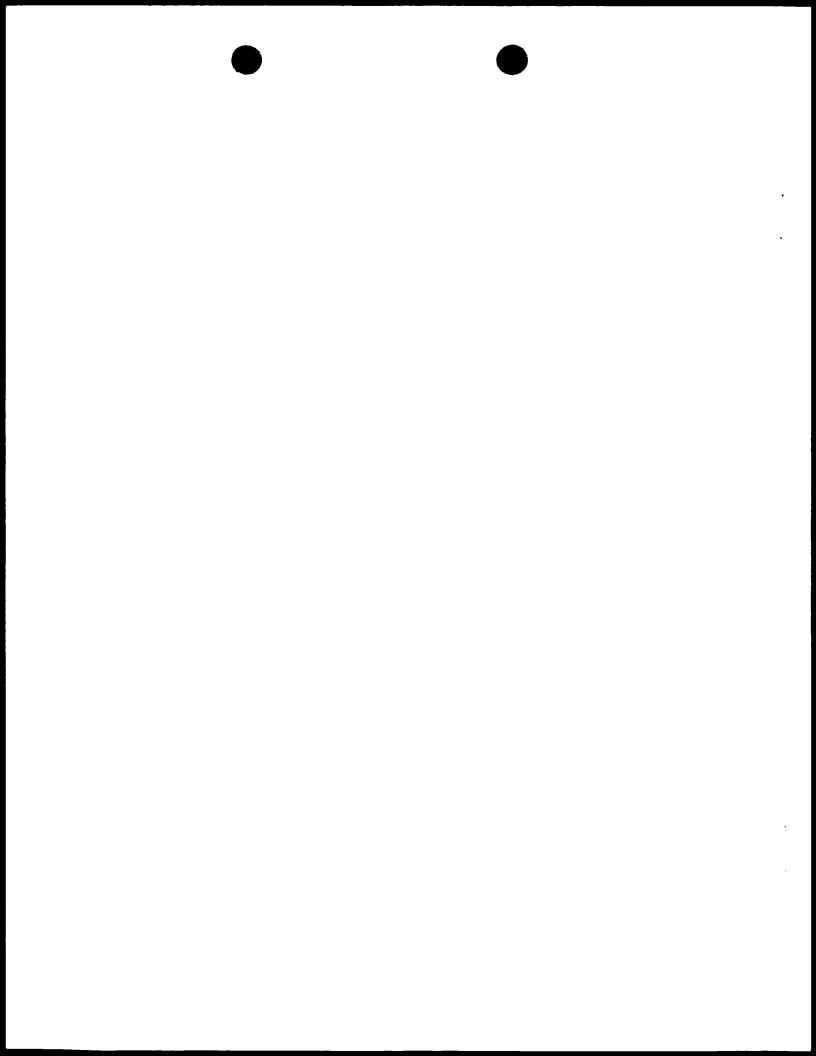
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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 99/00417

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   C12N15/12    C07K14/47    C07K14/705   C12N9/16     C12N9/64
        A61K38/17    A61K38/46    A61K38/47    C12N15/55    C12N15/57
        C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   C07K   C12N   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No |
|---|---|---|
| P,X | WO 98 43084 A (UNIV LELAND STANFORD JUNIOR) 1 October 1998 (1998-10-01) claims; table 4 --- | 1,5,6, 20-22,55 |
| X | WO 94 20127 A (CYTEL CORP) 15 September 1994 (1994-09-15) table 25 --- | 1,5,6, 20-22,55 |
| X | DE 195 16 673 A (PECHER GABRIELE DR) 31 October 1996 (1996-10-31) claims; examples --- -/-- | 1,13, 20-22, 43-45 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 30 December 1999 | 12/01/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Fuhr, C |

Form PCT/ISA/210 (second sheet) (July 1992)

1

page 1 of 2

## INTERNATIONAL SEARCH REPORT

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No |
|---|---|---|
| X | WO 97 08318 A (CORIXA CORP) 6 March 1997 (1997-03-06)<br><br>claims; examples<br>--- | 1,5, 20-22, 26,30, 31,34, 51-55 |
| A | WO 95 04548 A (JENNER TECHNOLOGIES) 16 February 1995 (1995-02-16) page 7, line 17 - line 35; claims; examples<br>--- | 1,26,32, 37,38,42 |
| P,A | DE 197 58 400 A (HANISCH FRANZ GEORG PROF DR ;MAX DELBRUECK CT FUER MOLEKULA (DE)) 1 July 1999 (1999-07-01) claims; table 1<br>--- | 1,26,32, 37,38,42 |
| X | WO 97 11715 A (AUSTIN RESEARCH INST ;SANDRIN MAURO SERGIO (AU); MCKENZIE IAN FARQ) 3 April 1997 (1997-04-03) claims; examples<br>--- | 1,11-13, 26-38, 46,51-55 |
| X | WO 97 35021 A (US HEALTH ;ZAREMBA SAM (US); SCHLOM JEFFREY (US); TSANG KWONG YOK) 25 September 1997 (1997-09-25)<br><br>claims; examples; table 10<br>--- | 1,5,20, 21, 26-38, 51-55 |
| A | WO 95 19783 A (CELIS ESTEBAN ;GREY HOWARD M (US); CYTEL CORP (US); KUBO RALPH T ()) 27 July 1995 (1995-07-27) claims; examples<br>--- | 1,26 |
| A | WEINSTEIN B: "CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS, PASSAGE" CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS,XX,XX, vol. 7, page 266-357 XP002032461 the whole document<br>----- | 23-25 |

# INTERNATIONAL SEARCH REPORT

International application No

PCT/IL 99/00417

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**Box I    Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority. namely:

   Remark: Although claims 37-38 are directed to a method of treatment
           of the human/animal body, the search has been carried out and
           based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:    43-45
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out. specifically:

   see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

---

**Box II    Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant. this International Search Report covers all
       searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee. this Authority did not invite payment
       of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant. this International Search Report
       covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
       restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**        [ ] The additional search fees were accompanied by the applicant's protest.

                             [ ] No protest accompanied the payment of additional search fees.

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Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

# INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION CONTINUED FROM     PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.:   43-45

Present claims 23-25 relate to an extremely large number of possible compounds/products. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds/products prepared in the examples and closely related homologous compounds like those mentioned in the sequence listing.

Present claims 43-45 relate to an extremely large number of possible compounds/products/apparatus/methods. In fact, the claims contain so many options, variables, possible permutations and provisos that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, no search has been carried out for this parts of the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

| Patent document cited in search report | | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|---|
| WO 9843084 | A | 01-10-1998 | US | 5858685 A | 12-01-1999 |
| | | | AU | 6570998 A | 20-10-1998 |
| WO 9420127 | A | 15-09-1994 | AU | 6359494 A | 26-09-1994 |
| | | | AU | 6597998 A | 02-07-1998 |
| | | | BR | 9406652 A | 10-09-1996 |
| | | | CA | 2157510 A | 15-09-1994 |
| | | | CN | 1118572 A | 13-03-1996 |
| | | | EP | 0703783 A | 03-04-1996 |
| | | | JP | 8507525 T | 13-08-1996 |
| | | | NZ | 263050 A | 24-11-1997 |
| | | | SG | 49008 A | 18-05-1998 |
| DE 19516673 | A | 31-10-1996 | NONE | | |
| WO 9708318 | A | 06-03-1997 | US | 5854206 A | 29-12-1998 |
| | | | AU | 7012496 A | 19-03-1997 |
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(54) Title: REMOTE-AUDITING OF COMPUTER GENERATED OUTCOMES USING CRYPTOGRAPHIC AND OTHER PROTOCOLS

(57) Abstract

A computer device and method for encoding a message corresponding to an outcome of a computer game, and a computer device and method for decoding the message to detect a fraudulent outcome. The computer device used to generate the encoded message includes a memory device containing encoding control code and a processor configured to process the encoding control code in conjunction with a computer game outcome (148) to generate an encoded message containing the computer game outcome (158) and to transmit the encoded message (154) to a human-readable output device, such as a display device. The computer device includes various tamper resistant or tamper evidence features. A secure module containing the processor and memory is used to plug into an existing personal computer or dedicated game device. The device also includes a system for metering use of a computer game.

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# REMOTE-AUDITING OF COMPUTER GENERATED OUTCOMES USING CRYPTOGRAPHIC AND OTHER PROTOCOLS

## BACKGROUND

### 1. Field of the Invention

The present invention relates generally to authentication of computer generated game or test results ("outcomes"), and more particularly, to a system by which persons who play games or take tests on a game or testing computer, respectively (hereinafter the "game computer" or "testing computer"), may submit the outcomes of the games or tests to a central authority having at least one central computer, and have the central computer "certify" those outcomes as being accurately reported and fairly achieved. This certification of the computer generated result constitutes a "remote-auditing" of the activity taking place on the game computer. In one application, the system enables computer generated game tournaments in which players play the games on game computers and compete against each other by submitting the outcomes for those tournament games to the central computer, which certifies the outcomes and rates and ranks the players. In another application, the system provides for players of computer games to obtain a certified ranking and rating without participation in a tournament. In other embodiments, the system provides for self-authentication and certification of outcomes for games played on the game computer by the game computer itself, or for mutual-authentication and certification of such outcomes on any other game computer in the system. The system further enables the submission and certification of test outcomes using the same methodology.

The present invention also provides for "pay-per-use" in the home video game environment, where any game computer may be turned into a video game arcade machine by metering usage of the game computer and/or game programs that run on the game computer. Players simply pay per game,

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or for play over a specified period of time in accordance with different pricing protocols. The invention also allows for "time-dependent disablement" which lets players acquire game consoles for a relatively low down payment. Charges for game play may then be incurred on a daily, weekly, monthly, or some other periodic basis.

## 2. Description of the Prior Art

Tournaments are a popular form of recreation and are amenable to many forms of organized activities, such as sports or games. There are two primary types of tournaments. In the first, players compete against one another (i.e., head-to-head), singularly or in teams, under controlled conditions. Examples include boxing, chess, karate and the like. In the second, players play a game without direct or simultaneous interaction with another player, where the player having the best score (e.g., golf, bowling, etc.), fastest time to completion (e.g., puzzles) or some combination thereof is pronounced the winner. Winners earn recognition for their skill and sometimes even prizes. Accomplished players of games of skill are often provided with an officially recognized ranking, rating and/or title.

Classic tournaments are usually held at a specific time and at a specific location, where they are conducted under a set of rules which apply equally to all contestants, and under the supervision of one or more judges and/or a sanctioning authority. A typical chess tournament may include one hundred to two hundred players who get together at a central location. They pay an entry fee and play a series of games over the course of a specified time period under the auspices of an officially sanctioned tournament director(s). At the end of the competition, the players are ranked and cash prizes are awarded to the top finishers. The United States Chess Federation administers a national rating system that ranks players with a numerical rating based upon the results of tournament sanctioned games against other rated players. Ratings may change over time as the player

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wins and loses games played in ongoing tournaments. Various rating ranges are given named titles. For example, an "Expert" chess player has a rating of between 2,000 and 2,200 and a "Master" chess player has a rating over 2,200 and so on.

5      The aforementioned tournaments have several drawbacks. Since most tournaments are held at some specified location, it is likely that some players may have to travel an appreciable distance, incurring expenses for travel, food, lodging and the like. Furthermore, it is often

10 difficult to schedule a given tournament at a time that is convenient for all participants. In addition, there are only a limited number of sanctioned tournament directors who are available to run such tournaments. Since the fundamental object of any tournament is to ensure the integrity and

15 authenticity of the results, without a tournament director, the results of the tournament are not verifiable. It is also difficult and impractical to run niche tournaments that appeal to a very small segment of the population, as the fixed costs associated with running a tournament can make it

20 economically impractical where only a few participants are involved.

     Aside from the so-called classic tournaments mentioned above, the players of many popular computer generated games enjoy competing for bragging rights as to

25 who has the best score. Most arcade gaming machines typically display a series of high scores identifying the most recent top scoring players who played on a specific machine. Similarly, some dedicated game systems such as Nintendo, Sega and the like, and personal computers with

30 dedicated game software, may display a series of high scores identifying top scoring players. While this enables a player who achieves a sufficiently high score to compare his or her performance with other players who have played on that particular machine or computer with that software, there is

35 no way to prevent players from lying to others about their

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"purported" score. Therefore, there exists a need for a
system whereby players of such games can register their
scores with a central computer that certifies the scores and
enables players to receive their ranking/rating with respect
to other players on a national or even worldwide scale. In
5   this connection, such a system could enable players of such
games to participate in "electronic" tournaments where
players either play individually or in teams on
independently disposed game computers, or head-to-head via
an on-line connection between at least two competing
10  players.

One approach to electronic tournaments is
disclosed in U.S. Patent No. 5,083,271 to Thacher et al.
("Thacher"). In the Thacher system, a plurality of gaming
terminals are networked to a central computer. A player
15  purchases credit, enters a tournament, and is assigned a
unique identification code. This identification code is
stored in the gaming terminal and at the central computer.
The player then proceeds to play a tournament game on the
gaming terminal. When the player has finished the game, the
20  player's score is transmitted to the central computer along
with the player identification code and a game
identification number. The central computer sorts through
all of the scores at the conclusion of the tournament and
determines a winner. The Thacher patent claims to provide
25  some level of protection against substitution of players by
utilizing a separate personal identification code for each
player. Thus, to the extent that a player's personal
identification code is not compromised, the person playing
the game is uniquely identified with the achieved score.
30  This arrangement has disadvantages, however, including the
extensive network between all of the participatory game
terminals, and the inability to verify that the scores in
the tournament games were accurately reported and fairly
achieved. For example, there is nothing in the Thacher
35  system which prevents a player from modifying the game

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software to produce a more favorable outcome, or from intercepting communications of score and identification data from the remote gaming terminal to the central computer and then altering the same to register a false score.

5      Another well known system for "authenticating" video game scores utilizes a primitive method in which players take photographs of both video screens containing game scores and the game console, and then mail the pictures to a central authority. The monthly magazine Nintendo POWER publishes the Power Players' Arena, in which top scoring
10     players are identified. Top scoring players receive Nintendo POWER Stamps which can be redeemed for prizes. The photograph of the video screen ostensibly prevents a player from simply making up a score. The photograph of the video screen and the game console supposedly enables the central
15     authority to determine whether the player has utilized any unauthorized device to change the standard play conditions for the game. This system has a number of disadvantages. Taking a clear photograph of a video display is often difficult due to the reflective nature thereof. There is
20     also a considerable amount of time that is required to transmit the photograph to the central authority and players must wait for the scores to be authenticated by Nintendo and thereafter published. This system is also vulnerable to players hacking the game software. No effort is made to
25     determine whether or not the game software was tampered with. The use of well known interposing devices such as the GAME GENIE, which fits into the access port of a standard game console and enables codes to be entered that temporarily change the play conditions as software
30     instructions are loaded into the read-write memory of the game console from the read only memory of the game cartridge, is ostensibly prevented by requiring that a photograph of the entire game console accompany the photograph of the video screen. However, players can easily
35     circumvent this problem by playing a game with an

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interposing device, taping the output with a VCR, thereafter removing the interposing device, and then playing back the recorded game for a subsequent photograph to be made without the interposing device installed in the game console.

5          Thus, there exists a need for a system that enables game computers operating independently at different times or in different places to certify their game outcomes in a manner that can be verified by a recipient thereof for purposes of comparison with other game outcomes. The system should allow such certification to be performed either by

10         another game computer, or by a central computer. The system should not require complicated networking or real-time connections between the game computers, or between each game computer and the central computer during game play. The system should further allow for establishing the players'

15         ranking, rating and/or title with respect to other players of the games based upon the players' certified scores.

           In view of the above, there also exists a need for a system which permits players to participate in tournaments on game computers at any place and any time, without

20         requiring complicated and costly networks or an on-line connection between the game computer and a central computer while the game is being played, without the need for the players to go to a specified location, and without the need to have an officially sanctioned tournament director present

25         where the games are being played while still ensuring the authenticity of the participants' scores. The system should further allow for establishing the players' ranking, rating and/or title in the tournaments with respect to other players of the games based upon the player's certified

30         scores.

           It is also known in the art to remotely control and monitor the use of video game software as disclosed in U.S. Patent No. 5,497,479 to Hornbuckle. This patent teaches a system whereby rental software is downloaded from a

35         central computer to a remote control module (RCM) which is

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operably associated with a game computer. The RCM operates to receive rental software packages from the central computer, and to control and verify the use of such software on the game computer. The rental software resides in the insecure memory of the game computer. A portion of the software is referred to as a "key module", a part of the software that is essential to program execution and without which the overall program will not execute on the game computer. The key module resides in an encrypted format, and must be decrypted by the RCM. After such decryption, the key module is loaded with the rest of the program into the RAM of the game computer for execution. When the program is terminated, the decrypted instructions are erased from the RAM of the game computer. The RCM records the elapsed time between starting and stopping of the rental program, and stores such information in its memory for subsequent processing.

The Hornbuckle system suffers a primary drawback in that the key module resides in the insecure RAM of the game computer, thereby enabling a hacker to get at the key module, and allowing replacement of the key module in the data storage of the game computer. It would therefore be desirable to provide a system in which the use of game programs can be metered using cryptographic protocols without compromising secure encrypted portions of the such programs by not loading the same in unencrypted format into the insecure memory of a game computer. It would also be desirable to provide a system in which use of the game computer itself can be metered using similar protocols.

## SUMMARY OF THE INVENTION

The present invention, in one aspect, is directed generally to a computer device including (1) a memory device containing encoding control code and (2) a processor configured to process the encoding control code in conjunction with a computer game outcome to generate an encoded message containing the computer game outcome and to

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transmit the encoded message to a human-readable output device, such as a display device. While this processor may also execute a computer game program, the computer device may also include a second processor which executes the computer game program. The computer device may also include various tamper resistant or tamper evidence features. In one embodiment, the processor and memory are part of a secure module which plugs into an existing personal computer or dedicated game device. The invention is also directed generally to a method including the steps of executing a computer game program to generate a computer game outcome, encoding the computer game outcome to generate an encoded message, and providing the encoded message to a user, who may then transmit the encoded message to a device configured for decoding the encoded message to reveal the computer game outcome. The invention in yet another aspect is directed generally to a central or host computer device having (a) a memory device containing decoding control code and an encoded message corresponding to a computer game outcome and (b) a processor configured to process the code to decode the encoded message to reveal the computer game outcome.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is an overall schematic of the inventive system in one embodiment;

FIG. 1B is an overall schematic of the inventive system in a self-authentication and mutual-authentication embodiment;

FIG. 2 is an overall schematic of the inventive system in another embodiment;

FIG. 3 is an overall schematic of the inventive system in still another embodiment;

FIG. 4A is schematic of the memory arrangement and general components of the game computer;

FIG. 4B is a schematic of a game cartridge in one embodiment;

FIG. 4C ia a schematic of a secure perimeter for

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the encryption/decryption module;

FIG. 4D is a schematic of a game cartridge in another embodiment;

FIG. 4E is a schematic of a game cartridge in still another embodiment;

FIG. 4F is a schematic of a game cartridge in yet another embodiment;

FIG. 4G is a schematic of an embodiment utilizing a secure perimeter and VCR in connection with a game console type game computer;

FIG. 4H is a schematic of the secure perimeter/VCR interface;

FIG. 5 is a flow-chart of various Authenticatable Outcome Message generation protocols;

FIG. 6A is a schematic of an exemplary software integrity check;

FIG. 6B is a flow chart of the software integrity check in the embodiment depicted in FIG. 6A;

FIG. 7 is a schematic of an exemplary memory arrangement and some hardware for the central computer;

FIG. 8A is a flow-chart of an exemplary tournament entry procedure;

FIG. 8B is a schematic of an arcade implementation;

FIG. 9 is a flow-chart of game play;

FIG. 10A is a flow-chart of an illustrative outcome submission and certification sequence;

FIG. 10B is a flow-chart of an illustrative biometric verification procedure;

FIG. 11 is a flow-chart of a challenge/response protocol;

FIG. 12 is a flow-chart of a Broadcast Start Message sequence in one exemplary embodiment for races of skill; and

FIG. 13 is a flow-chart of an exemplary tournament sequence for head-to-head games;

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FIG. 14 is a schematic of a meter for enabling pay-per-use game play in accordance with the present invention;

FIG. 15 is a schematic of metered software for use in a pay-per-use embodiment;

5    FIG. 16 is a flow chart of an initialization protocol for the meter;

FIG. 17 is a flow chart of an adding a new program protocol for the meter;

FIG. 18 is a flow chart of an authorization from
10   the central computer protocol for the meter;

FIG. 19 is a flow chart of an updating cost information protocol for the meter;

FIG. 20 is a flow chart of a synchronizing clock protocol for the meter;

15   FIG. 21 is a flow chart of a starting metered software protocol for the meter;

FIG. 22 is a flow chart of a running metered software protocol for the meter;

FIG. 23 is a flow chart of a reporting usage
20   protocol for the meter;

FIG. 24 is a flow chart of an auditing protocol relating to pay-per-use;

FIG. 25 is a flow chart of an outcome authentication protocol using the meter;

25   FIG. 26 is a flow chart of another outcome authentication protocol using the meter;

FIG. 27 is a schematic of a descrambling pay-per-use embodiment; and

FIG. 28 is a schematic of a metering device
30   incorporated into a video game controller.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

With reference to the several views of the drawings, there are shown several embodiments of a system in accordance with the present invention generally denoted by
35   the reference numeral 10.

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In the embodiment shown in FIG. 1A, the system is principally comprised of a central computer 12 associated with a central authority, and a plurality of game computers 14. The term "game computer" is intended to include personal computers ("PCs"), personal digital assistants, coin-operated arcade video gaming machines, television units coupled to game units (e.g., game consoles such as Nintendo, Sega, etc.) and portable game devices (e.g., GAME BOY, GAME GEAR, NOMAD and the like). For the purpose of description, the game computer depicted in the drawings replicates a standard PC. Each game computer 14 contains software and/or firmware (for convenience, all references herein to programs are to "software") which generates games of the type well known in the art. The practice of playing games on the game computer 14 may be classified as a person against game activity. At the conclusion of a game, the player submits the outcome (e.g., a score and/or time to completion, a combination thereof and any other play-related data) to the central computer 12 as described in detail below. However, many popular games allow for players to play against each other on the same game computer 14, or by establishing an on-line connection between the players' respective game computers 14. In this case, the outcome of the players' competition is submitted and certified (e.g., player A beat player B with a score of X to Y). In the following description, each game computer 14 includes game software 15 which resides in memory generally identified by the reference numeral 23. The memory 23 includes read-write memory RAM, read-only memory ROM, and any non-volatile data source of programs associated with the game computer 14, such as a game cartridge, hard-disk, CD-ROM, PCMCIA card or special flash ROM chip. The specifics of the game software 15 as relates to the present invention will be described in more detail below. The game computer may also have an associated input control device 17, such as a joystick (shown) as is well known in the art. The input/output device

17 may comprise multiple joysticks or controls for players to play against each other.

The central computer 12 authenticates and/or certifies outcomes and manages tournaments. It is shown schematically in the drawings as a single unit, but may comprise a plurality of networked computers as required. In order to facilitate tournaments on a large scale, it may be required that the central computer 12 be broken up into regional computers, national computers and even a top level international computer. These computers may be interconnected via data networks such as TYMNET, DATAPAC or the like. The national computers poll the regional computers and the international computer polls the national computer for tournament data. Thus, regional tournaments only utilize the regional computers. If national tournaments are to be held, the national computers obtain the required tournament information from the regional computers. In the case of an international tournament, the international computer polls the national computers. Alternatively, several computers on a single level may be arranged so as to periodically verify each other in accordance with known principles. There are many ways in which the computers can be arranged, so for the purpose of description herein the central computer 12 is referred to as a single unit. As described in more detail below, in another embodiment of the invention, each game computer 14 is capable of authenticating an outcome from another game computer 14, with the central computer 12 operating as a Key Distribution Center ("KDC"), i.e., a database of encryption keys used for authenticating messages. The central computer 12 may also operate a "central scoreboard," i.e., a database where all certified scores and statistical information on players and teams are maintained. Statistics for a given player may include information on opponents, the time of play, ratings, rankings and the like. The information may be publicly available, or password protected (i.e., available only to

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those persons with the proper access password, or to those that have attained a certain rating threshold)  The information may be made available via the Internet or major online providers, downloaded to game computers, or by mail or telephone.

In the embodiment shown in FIG. 1B, the game computers 14 are capable of "self-authentication" and "mutual-authentication."  Self-authentication means that a game computer 14 can authenticate an outcome incorporated in an Authenticatable Outcome Message AOM which it generated.  For example, if a player claims that a score printed on a piece of paper, or stored in a given memory media, is authentic, and this score is embodied in an Authenticatable Outcome Message AOM, the score may be authenticated by authenticating the authenticatable message AOM on the game computer 14.  Similarly, the authenticatable message may be authenticated on any other game computer 14 in the system (i.e., mutual-authentication).  The authentication protocols will be explained in more detail below.

Referring again to the embodiment shown in FIG. 1A, at least one Interactive Voice Response Unit ("IVRU") 16 is associated with a telephone network and coupled to the central computer 12 through a standard interface for access from a plurality of telephones 18, to enable players to enroll in tournaments and/or to submit the outcomes of the games to the central computer 12 for certification.  In certain implementations, a player may register personal information and/or the game software 15 with the central computer over the telephone 18.  Specifically, IVRUs are responsive to both voice and touch-tone signals communicated from the telephones 18.  In this connection, the game computer 14 may communicate with a Dual Tone Frequency Modulator ("DTFM") to generate messages compatible with the IVRUs 16. An acoustic coupler 115 may be used to receive messages from the telephone 18 in the same manner.  Since the operation of the IVRUs 16 and DTFMs are well known in

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the art, they need not be described in detail herein. The IVRUs 16 may be associated with an automatic call distributor ACD of the type known in the art to balance the call load. During times of peak calls, calls to any IVRU 16 may be routed to a neighboring IVRU 16.

5          In an alternative embodiment shown in FIG. 2, the game computers 14 may communicate with the central computer 12 via a modem 20. In this regard, the game computers are not considered to be on-line with the central computer during the game. When a player desires to submit his or her

10        outcome for a particular game or time of completion for a race of skill, the game computer 14 dials up and obtains access to the central computer, and uploads the game outcome information. This is discussed in more detail below. In this connection, it is anticipated that the central computer 12

15        may be accessed via a website 22 on the Internet 24 or over an on-line data network including commercial on-line service providers, bulletin board systems and the like, as shown schematically in FIG. 3. The process for establishing an on-line connection to a website on the Internet is well

20        known and need not be described here in detail.   It is essentially analogous to establishing a direct on-line link between the game computer 14 and the central computer 12. In yet other embodiments, the game computers 14 may communicate with the central computer 12 over RF, cable TV, satellite

25        links and the like. For example, in an RF embodiment, communications are simply broadcast in an RF format and transmitted between the game computer 14 and the central computer 12. The same prompting arrangement as with an IVRU 16 may be employed, with the player entering commands

30        instructing the game computer 14 to send a message to the central computer 12 directly through the key pad or joystick of the game computer 14. Similarly, messages may be communicated over a cable TV link directly to a television interfacing with a game console.

35        It is also anticipated that communications between

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the game computers 14 and the central computer 12 can be implemented with a physical data memory device such as a smart card, diskette and the like. The game computer 14, for example, might store game-related data onto a diskette which the player would be required to mail to the managing authority for inspection at the central computer 12. Such a procedure might be required in all instances where the player had won a substantial prize, or where cheating is suspected by tournament officials. Moreover, the game computer 14 may communicate with a printer for printing a copy of an outcome, a game screen containing the outcome and any other relevant data such as game statistics and the like, which may be mailed or faxed to the central authority for subsequent certification of the outcome and such data with the central computer 12.

Referring now to FIG. 4A, there is shown a schematic of a portion of an illustrative memory arrangement and some hardware for the game computer 14 in the system of the present invention. For convenience, the internal memory 23 of a personal computer 14 is shown. As described above, the memory 23 includes RAM and ROM, and is coupled to a central processing unit ("CPU") 27 in a conventional manner. The CPU 27 and related hardware are typically referred to as a processor. We use the term "associated memory" to indicate that the game computer memory 23 may also be defined to include a non-volatile insecure data source of programs such as a game cartridge, hard disk, floppy disk, PCMCIA card, special flash ROM chip and the like. Secure memory is disposed within a secure perimeter that will be defined below. The processor loads programs into RAM and executes programs from memory in a conventional manner. In the illustrative embodiment, memory 23 contains a game software package 15 comprised of a game program 26, an encryption/decryption module 28, a transmission error check module 30, a secret software or game computer ID ("SSCID") stored in memory area 32 which uniquely identifies the

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particular game software 15 and/or game computer 14, a
time/date module 33, and biometric data in memory area 35.
The game software 15 may comprise a single "program," with
the individual elements thereof constituting separate
routines. For the purpose of description herein, the term
5    game software 15 can be broadly defined to include a
plurality of constituent programs, instructions, routines,
files, databases, etc. The game software 15 may also have an
associated non-secret software serial number SSN, the
purpose of which will become apparent below. The
10   transmission error check module 30 is used to process all
incoming messages to the game computer 14 to detect manual
inputting errors, corruption of transmitted data due to
communication problems such as line noise and the like, to
enable a resend indication or request to be made. The
15   time/data module 33 time-stamps messages using signals from
the clock 36. The biometric data stored in memory area 35 is
used for player verification, which is described in greater
detail below. A dedicated game computer 14 may have all of
its components including its associated memory 23, CPU 27
20   and clock 36 housed in a tamper-resistant and/or
tamper-evident enclosure to prevent and reveal,
respectively, tampering with any of these components.
Tamper-evident enclosures include thermoset wraps which,
upon inspection, can reveal any attempt to physically open
25   the structure. Tamper-resistant structures may
electronically destroy the memory contents of data should a
player try to physically open the structure. A secure
perimeter is a defined physical area of hardware which is
tamper-resistant and/or tamper-evident, as described in more
30   detail below.

The game program 26 generates games of skill of
the type known in the art and commonly played in tournaments
such as chess, backgammon, bridge, and the like. Other
well-known games of skill (e.g., SONIC AND KNUCKLES,
35   VECTORMAN, DONKEY KONG COUNTRY, MORTAL KOMBAT, STREET

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FIGHTER, etc.) include those played on dedicated gaming machines such as game consoles, in an arcade or other place where such gaming machines reside. The game program 26 may be configured to enable games to be played in a practice mode, in which the outcomes are not certified or part of a tournament. Such practice games may not have the full functionality of tournament games. A practice golf game, for example, might have less complex wind patterns -- with wind speed and direction being fixed for a given hole. The tournament version may have winds that frequently change, and which may vary depending on the location of the ball. The game program 26 may also be arranged to include teaching modes for instructing players in a manner consistent with the way they play the game or its result.

The game program 26 may compile a statistical database 31 to store tournament game data that specifically relates to the player's actions during the game. For example, the player of a tournament game may have found X treasures, reached Y levels and eliminated Z enemies. This information may be stored and accessed only by the player who enters the proper code or message into the game computer 14. This message may be the start message which enables tournament play as discussed below. In a further application, certain aspects of the game, such as, for example, a screen or sequence of events where a player performed a certain move or where a particular opponent was defeated, may be stored and indexed in a database by a certain code to enable the player to call up any one of such screens or sequences at a later time by entering the start message associated with that game (in the case of a tournament) or by some other special command. A menu can be generated upon receipt of the start message or command, enabling the player to select and view the desired screens or particular sequences of events in the game.

The game program 26 may generate races of skill. These include puzzles where the player having the quickest

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time to completion is declared the winner. A crossword
puzzle is a classic race of skill in which players compete
to be the first to correctly solve the puzzle. Driving games
with lap times also represent races of skill in which the
shortest time to the finish line is declared the winner.

5     Referring again to FIG. 4A, to time races of skill, the game
program 26 may use a signal from the computer's clock 36
through the time/date module 33 to time-stamp a particular
outcome message or to generate a time message which
represents the amount of time the player took to complete a

10    given game. In this connection, the clock 36 may be housed
within a tamper-resistant and/or tamper evident seal 38.
Preferably, a real-time clock 36' is disposed within a
secure perimeter 300 as described below. In another
embodiment described below, the clock 36' may reside within

15    a dedicated game cartridge 21.

In yet another application, the game program 26
may generate games of chance where the outcomes of such
games are submitted and certified in accordance with the
invention.

20    The outcome of a game is defined as the entire set
of the results of the game, including a score, time to
completion in the case of a race of skill, or a combination
of both. Alternatively, the outcome may be comprised of all
data relating to the game itself (i.e., data stored in

25    memory that enables the entire game to be recreated). In a
golf game, for example, such data may include each shot that
the player takes, which represents a combination of
parameters such as current wind speed, club selected, foot
placement, force with which the ball is hit, etc. If these

30    parameters are stored to a disk as the game proceeds, it is
possible to subsequently recreate the entire game by
replaying the stored parameters. For added security, these
values may be stored in encrypted form so that the player
cannot alter the game data representing such results after

35    the game is completed.

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For typical scored games, execution of the game software 15 by the game computer 14 results in an outcome representing the player's score and, optionally, additional game related information such as the number of levels attained, amount of time spent at each level, number of lives lost, number of enemies eliminated and the like. A game may also have multiple outcomes associated with it. In a game of chess, for example, each move may be considered a separate outcome. Each move can be authenticated by the central computer. In some games, an individual's score may be dependent upon the scores of other players. Authenticating one player's outcome thus requires knowing the outcomes of the other players. For a race of skill, such as a puzzle, execution of the game software 15 by the game computer 14 results in an outcome representing the elapsed time it took the player to complete the game and, optionally, other game related information or subsidiary events such as the completion of certain sub-levels in the game and the like.

An outcome may be transformed or incorporated into an Authenticatable Outcome Message AOM (for clarity, a time of completion is transformed or incorporated into an authenticatable time message ATM) by using a variety of cryptographic protocols including one-way hash functions (also known as compression functions, contraction functions, message digests, fingerprints, cryptographic checksums, data integrity checks (DICs), manipulation detection codes (MDCs), and data authentication codes (DACs)), one-way hash functions with encryption keys (also known as message authentication codes (MACs)), digital signatures, and the like, with the encryption/decryption module 28. The practice of using cryptographic protocols to ensure the integrity and security of messages is well known in the art and need not be described here in detail. For reference, one of ordinary skill in the art may refer to BRUCE SCHNEIER, APPLIED CRYPTOGRAPHY, PROTOCOLS, ALGORITHMS, AND SOURCE CODE IN C,

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(2d Ed, John Wiley & Sons, Inc., 1996). The encryption/decryption module 28 contains algorithms and keys for encrypting, decrypting and/or authenticating messages. Examples of well-known cryptographic authentication protocols are as follows:

5
<u>Encryption</u>:

Setup: Central computer 12 and game computer 14 share a secret key.

1. Game computer 14 encrypts outcome message with the
10  shared secret key to form an Authenticatable Outcome Message AOM.

2. Communicate Authenticatable Outcome
15  Message AOM to central computer 12.

3. Central computer 12 reads and decrypts the Authenticatable Outcome
20  Message AOM with the same key.

4. If the message is intelligible, then the central computer 12 accepts
25  the outcome message as authentic.

*Encryption may be implemented with an algorithm such as DES (U.S. Government
30  standard, specified in FIPS PUB 46). Encryption may utilize any of several algorithms known in the art such as IDEA, Blowfish, RC4,
35  RC2, SAFER, etc. See APPLIED

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*CRYPTOGRAPHY.*

<u>Message Authentication Code</u>:

Setup: Central computer 12 and game computer 14 share a secret key.

1. Game computer 14 hashes outcome message with a MAC and the shared secret key to form an Authenticatable Outcome Message AOM.

2. Communicate Authenticatable Outcome Message AOM to central computer 12.

3. Central computer 12 reads the AOM and hashes the message with the shared secret key.

4. If the generated hash matches the received hash, the central computer 12 accepts the outcome message as authentic.

*Any of the MAC algorithms, such as, for example, DES, CBC and the like may be applied in this application.

<u>Encryption with Public Key Cryptography</u>

Setup: Game computer 14 has a public-key/private key pair. The central computer 12 knows the game computer 14's public key.

1. Game computer 14 encrypts outcome message with the private key to form an

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Authenticatable Outcome Message AOM.

2. Communicate Authenticatable Outcome Message AOM to central computer 12.

3. Central computer 12 decrypts the AOM with the public key of the game computer 14.

4. If the message is intelligible, the central computer 12 accepts the outcome message as authentic.

A sample algorithm for this procedure is RSA.

Signing with Public Key Cryptography

Setup: Game computer 14 has a public-key/private key pair. The central computer 12 knows the game computer 14's public key.

1. Game computer 14 signs the outcome message with the private key to form an Authenticatable Outcome Message AOM.

2. Communicate Authenticatable Outcome Message AOM to central computer 12.

3. Central computer 12 verifies the signature using the outcome message and the public key. The mathematics of verification indicates whether the outcome message is authentic.

4. If the outcome message is intelligible, then the central computer 12 accepts the outcome message as authentic.

There are several ways to ensure that an Authenticatable Outcome Message AOM is "fresh" (i.e., it has been used more than once). In the first, known as "challenge/response", the central computer 12 generates a random or sequence number (also referred to as a "nonce") and communicates it to the game computer 14. The game computer 14 then incorporates this random number in the

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Authenticatable Outcome Message AOM. If the random number received matches the random number just generated, the central computer 12 accepts the message as fresh, i.e., an old message would contain a different random number.

In another method, the game computer 14 includes a sequence number in the Authenticatable Outcome Message AOM. This sequence number is incremented by one every time the game computer 14 generates an Authenticatable Outcome Message AOM. The central computer 12 stores the most recent sequence number in memory. It accepts the current outcome message if the sequence number received is one greater than the stored sequence number.

In yet another implementation, the game computer 14 includes the current time in the Authenticatable Outcome Message AOM. The central computer 12 then checks the time associated with the Authenticatable Outcome Message AOM against the time from the central computer's associated clock. If the times are within a prescribed window, the current outcome message is accepted as fresh.

In still another procedure, the game computer 14 includes a random number in the Authenticatable Outcome Message AOM. The central computer 12 maintains a database of all random numbers received from the game computers 14. If the new random number is not in that database, then the current Authenticatable Outcome Message AOM is accepted as fresh. If a time element is incorporated as well, then the central computer 12 only has to store a relatively small quantity of unexpired messages.

In FIGS. 4A and 4B, the encryption/decryption module 28 is depicted as part of the game software 15. In that embodiment, the encryption/decryption module 28 refers to cryptographic algorithms and keys or other data, software instructions and the like. These reside with the rest of the game program in a data storage device such as a game cartridge, hard disk, CD-ROM or the like. The actual processing to implement the cryptographic protocols takes

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place in the game computer's CPU 27, not within a specialized cryptographic processor.

Preferably, as shown schematically in FIG. 4C, some or all of the encryption/decryption module 28 resides within a secure perimeter 300. A secure perimeter is a defined physical area of hardware which is tamper-resistant and/or temper-evident, in which resides data or algorithms whose characteristics must not be alterable in order for a system to remain secure. Examples of devices which incorporate secure perimeters include U.S. military encryption devices such as the STU-III telephone made by Motorola and AT&T, and the iPower card, available from National Semiconductor Corp. The latter is a dedicated encryption/decryption device embodied in a PCMCIA card which can interface with the game computer 14 through, for example, the game computer's PCMCIA socket (for game computers 14 with PCMCIA compatibility). In the iPower card, both the key/data storage and cryptographic functionality are located within the secure perimeter. Referring again to FIG. 4C, a secure perimeter such as an iPower card, includes a 32-bit CPU 302 with ROM 304 containing encryption algorithms, a real-time clock 36' and an interface with an off-chip battery (310) - backed RAM 308 which holds encryption data and key information. Any attempt to tamper with or get at the encryption data stored within the device results in a memory loss of that data. Moreover, the I/O pins 312 of the device are electrically isolated to prevent pin-level probes, and the chip itself contains mechanical and chemical protection to prevent chip-probing equipment from accessing the encryption information from the [processor] CPU 302 directly. Such a secure perimeter 300 may additionally include additional non-volatile memory 313 for storing other software instructions and/or additional game related or other data as will be explained in more detail below. Hereinafter, the CPU 302 within the secure perimeter 300 will be referred to as the secure CPU 302, and

the memory within the secure perimeter 300 will be referred to as the secure memory. Thus, within an iPower card, all encryption/decryption functions are performed in the secure perimeter and do not take place in the CPU 27 of the game computer 14. Communications between the secure CPU 302 of the secure perimeter 300 and the CPU 27 of the game computer 14 are known in the art and need not be described here in detail. When the secure CPU 302 of the secure perimeter 300 is called upon by the game computer 14 to generate an Authenticatable Outcome Message, authenticate an Authenticatable Outcome Message, and/or perform any other required functions, the CPU 27 of the game computer 14 sends the appropriate signals to the secure CPU 302 of the secure perimeter 300. In this regard, the entire encryption/decryption module 28 is said to reside within the secure perimeter 300. This means that all cryptographic keys and algorithms are stored in memory within the secure perimeter 300, and all cryptographic functions are implemented on the secure processor 302 within the secure perimeter 300. Thus, when cryptograhic protocols are to be used for encryption and/or authentication, the game computer CPU 27 communicates commands and data to be encrypted or made authenticatable to the secure processor of the secure perimeter 300 as described below, requesting that the data be cryptographically processed. The secure CPU 302 of the secure perimeter 300 may be used to subsequently authenticate the Authenticatable Outcome Messages AOMs that it generates, as well as Authenticatable Outcome Messages AOMs from any other game computer 14 in the system. It may also be used to time-stamp messages or track times to completion for races of skill with the clock 36'. The secure CPU 302 may also perform some of the computational tasks required to execute the game software.

In an alternative embodiment, cryptographic keys may be stored in secure memory within the secure perimeter 300, but cryptographic algorithms and software instructions

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are stored in unsecure memory associated with the rest of
the game software 15 (i.e, in a conventional game cartridge,
hard disk, floppy disk, CD-ROM or the like), and actual
processing to implement the cryptographic protocols takes
place in the game computer's CPU 27.

5          External secure devices such as the aforementioned
iPower cards can function as "tokens." A token is a physical
computing device used by individuals to gain access to
protected electronic resources. Tokens commonly include
cryptographic capabilities and can store keys or other data.

10  Intelligent security tokens may be utilized to prevent
unauthorized player access to the game computer 14, as well
as for implementing the encryption/decryption functions for
outcome authentication and certification. The iPower card
described above, is an example of a token contained within

15  a secure perimeter.

Other such tokens include the SMARTDISK,
manufactured by SmartDisk Security Corporation. The
SMARTDISK contains a CPU and memory used for encrypting and
decrypting data. Thus, as with the iPower card, the

20  encryption/decryption module 28 may reside in the SMARTDISK
(i.e., all cryptographic functions are implemented within
the SMARTDISK). The SMARTDISK requires a user password to
function. Thus, access to the system requires the player to
physically possess the token and know the proper password.

25  Smart cards are similar tokens. They are shaped like
credit-cards, but contain an embedded microprocessor for
implementing various security functions.

Another type of token called TOUCH MEMORY is
manufactured by Dallas Semiconductor Corporation. This

30  device consists of a computer chip housed within a small
button shaped stainless steel case. The case may be
ring-shaped and worn around a player's finger. The chip
contains up to 64kb of RAM or EPROM, sufficient to store a
plurality of cryptographic keys. The device transmits data

35  bidirectionally at 16.3kb per second when placed into

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contact with a reader device. Each chip contains a unique serial number that is laser-etched into the chip at the time of manufacture. Keys from the device may be used in any of the cryptographic protocols described herein for authentication and/or encryption, as well as for user

5    identification. The DS1422 UNIQUEWARE product can be configured to transparently decrement each time that the device is used, allowing players to obtain and store a limited number of start messages, for example. The DS1427 configuration includes a tamper-resistant real-time clock

10   36' that may be utilized in the different applications described herein.

In yet another embodiment, a player may obtain a joystick which has a unique identifier associated with it. The joystick identifier may be used as a key in the

15   cryptographic protocols described herein, or to enable the player's game software 15 to generate a certified or tournament game. The key may be stored on a ROM chip within the joystick. When the game software 15 loads instructions to generate the game into the RAM of the game computer 14,

20   the key from the joystick is loaded into RAM and verified. If the proper key is not found, the game software 15 may be disabled. This is conceptually similar to a "dongle." Alternatively, the joystick may have an associated input/output interface for accepting data from and

25   communicating data to a secure perimeter such as an iPower card. Thus, the authentication protocols may take place within the structure of the joystick. This approach is described in more detail in an alternative embodiment below.

30   Other secure devices include the SENTINEL SUPERPRO manufactured by Rainbow Technologies. The SENTINEL SUPERPRO is a dongle which stores keys required for the operation of software applications. The software directs the computer it is running on to access the dongle and, if it does not find

35   the right key, suspends execution of the program.    The

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° dongle plugs into the parallel port or ADB port of a personal computer and measures 1.65 inches long by 2.125 inches wide. It contains 128 bytes of read/write memory organized as sixteen 64 bit words. These words can be used as time counters for leased software, or they can count

5 executions for limiting the operation of demonstration products. Memory cells can also be programmed with customer information, serial numbers, passwords, and the like. With regard to the present invention, the keys and algorithms [used by the] that form part of the encryption/decryption

10 module 28 could reside in the memory of such a dongle.

Dongles may also include specialized cryptographic processors and memory, allowing functionality similar to that found within an iPower card. Dongles configured to plug into a parallel port, however, have the advantage of being

15 compatible with nearly all Intel-based computer hardware, as opposed to iPower cards which require PCMCIA capabilities.

The above secure devices are particularly well suited to the storage of game related data for auditing purposes. In a computer golf hole-in-one tournament, for

20 example, it may be desirable to track each swing that a player takes since large prizes for a hole-in-one would attract hackers interested in forging such an event. To prevent such cheating, game parameters (swing speed, club used, etc.) would be sent to the secure CPU 302 where they

25 would be encrypted. This encrypted data could be stored in the non-volatile secure memory 313 within the secure perimeter 300 as an encrypted receipt file. Any player scoring a hole-in-one could be required to send in the secure device before receiving payment, allowing tournament

30 officials to examine the game data to see if it matched the claimed result. Alternatively, the encrypted game parameters could be communicated back to the central computer 14 and stored on the hard drive or copied to a floppy disk (insecure memory). In the event of a claim for a large

35 prize, the player would simply mail in the disk to the

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managing authority and the encrypted data would be decrypted and analyzed by the central computer 12 by recreating the game with such data to determine whether the claimed score was actually achieved.

For an additional level of security, the secure
5   CPU 302 may perform some of the game calculations normally executed by CPU 27. In an illustrative application, the game program renders a golfing game of skill, such as, for example, PGA TOUR 96 available from ELECTRONIC ARTS. In this game, a digital image of a golf game is rendered on the game
10  computer 14, comprising a golf ball on a tee, fairway, trees, sandtraps, etc. A human figure is superimposed on this background, and swings a golf club in response to player inputs via a keyboard or joystick. The player's club swing data represents various parameters, including the club
15  selected (e.g., one iron, two iron, three wood, etc.) and its specific characteristics (e.g., club head orientation), foot placement, and swing force, speed, direction and the like. In the course of a typical computer generated golf game, these parameters are applied to software instructions
20  that compute a trajectory path for the ball to generate a resultant ball location. After the player swings the club, the display may depict the new ball location relative to the hole. Other factors, including ambient conditions such as wind speed and direction or other random variables, may be
25  introduced for greater realism. With a secure CPU 302, the calculation as to the new location for the ball may be taken away from CPU 27. This is accomplished by making these calculations part of a Secure Software Component 710 of the game program 26, which will only run on the secure CPU 302,
30  for example, by encrypting the block of software instructions that relate to computation of such portions of the game with the above-described game parameters, or by requiring additional data or algorithms stored in secure memory within the secure perimeter 300 to make such
35  computation. Thus, the secure CPU 302 computes the new ball

position, which is then communicated back to the CPU 27 of the game computer 14 where it is displayed on screen. The results of the calculations can also be stored on the hard drive of the game computer 14 as described previously. Some game variables, such as wind speed, could also be generated
5   by the secure CPU 302. These variables would impact the calculation of the new ball position, and would prevent players from using mechanical devices to play a perfect game since at least one variable cannot be controlled. Alternatively, a game parameter such as wind speed, could be
10   generated by the secure CPU 302, and then transmitted to the CPU 27 where the new ball position could then calculated. This embodiment is described in more detail below with regard to the pay-per-use metering system.

For game console applications, a secure CPU 302
15   within a secure perimeter 300' may be adapted to interface with a VCR unit 400 as shown schematically in FIGS. 4G and 4H, to enable cryptographically protected recording and playback of games generated on the game computer 14. In this embodiment, the video output signal from the game computer
20   14 is communicated to a video input on the VCR 400, and a video output signal from the VCR 400 is communicated to a television 402 in a conventional manner. The secure CPU 302 and associated hardware within secure perimeter 300' [is] are configured to fit into a standard VCR slot 404. In
25   addition to the secure CPU 302, the secure perimeter 300' includes ROM 304 containing encryption algorithms, a real-time clock 306 and an interface with an off-chip battery (310) - backed RAM 308 which holds encryption data and key information. The secure perimeter 300' further
30   includes interface circuitry 406 for communicating signals from the read/write head 408 of the VCR 400 via an analog/digital ("A/D") converter 410 to the secure CPU 302. Video information is typically communicated to the television 402 in an RF format. The RF video signal may be
35   processed in the VCR 400 by the front-end receiving

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circuitry 412, which demodulates the video signal to a
base-band signal as is well-known in the art. Normally, the
demodulated information is what is recorded on a VCR tape
cassette. In the inventive application, the base-band video
signal data is converted to digital format by the A/D
5   converter 410, encrypted with a private key, and stored in
the non-volatile memory such as an EPROM 414. For playback,
the secure CPU 302 authenticates the game data, for example
by decrypting the data with the corresponding public key,
and the authenticated game data is then processed to
10   generate a video signal. The secure perimeter 300' may also
contain software instructions in ROM for generating an
Authenticatable Outcome Message AOM to be used as described
hereinbelow. This Authenticatable Outcome Message AOM may be
included in the video signal to appear on the television
15   screen at the end of the game.

Referring now to FIG. 4B, there is shown a
schematic of a game cartridge 21 for use with the system of
the present invention. The game cartridge 21 includes a
housing 19 that contains the game software 15 in a ROM 23a
20   built into the cartridge, and the ROM 23a interfaces with
the game computer 14 via an I/O interface 25 in a
conventional manner. The software serial number SSN may be
displayed on the exterior of the cartridge housing 19 as
shown. The game software 15 in the case of typical games
25   such as those offered by Sega and Nintendo, includes a game
program 26 which offers the player a choice of a tournament
enabled game or a non-tournament enabled (regular) game.
Tournament enabled games may be generated with the "cheat
codes," typically used by developers in testing the game,
30   disabled in the game program 26. In addition, certain play
aspects of the game which usually occur in some known
sequence or have some known characteristics (such as the
location of bonuses or certain challenges), may be changed
in the tournament version of the game to ensure that the
35   game is less predictable than that of the regular version.

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While the regular version of a computer golf game may have only a few sand traps, the tournament version may have many. Opposing pitchers may throw the ball at 80 miles per hour in a regular computer baseball game, while in the tournament version opposing pitchers throw at 100 miles per hour. Game cartridges may also contain game software 15 configured for one-time or limited time use.

Referring now to FIG. 4D, a game cartridge 21 contains the game software 15 in volatile memory 23b. The volatile memory 23b is connected to the I/O interface 25 in a conventional fashion. The volatile memory 23b is also connected to a power source 27 via a tamper switch 29. The tamper switch 29 is coupled to the cartridge housing 19, at the interface shown schematically at 31, so that any attempt to break open the cartridge housing 19 causes an interruption in power from power source 27 to volatile memory 23b, thereby causing all program data stored in volatile memory 23b to be erased. The tamper switch 29 may take many forms, depending upon the configuration of the game cartridge 21. In an exemplary embodiment, the tamper switch 29 is adapted to the cartridge housing 19 such that a physical incursion simply causes the tamper switch 29 to open. Alternatively, the tamper switch 29 may consist of a photocell sensitive to a certain level of light that causes a power interruption if the cartridge housing 19 is opened. In either case, an interruption of power to the volatile memory 23b causes all stored program data to be erased. This procedure is well-known in the art for securing computer memory devices. The clock 36 may also be housed within the game cartridge 21 such that any attempt to alter the clock 36 results in a loss of program data stored in volatile memory 23b.

Referring now to FIG. 4E, all game software data (excluding the encryption/decryption module 28) is encrypted and stored in non-volatile memory 23c, while the encryption/decryption keys and algorithms

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(encryption/decryption module 28) are stored in volatile memory 23d. Thus, any action which triggers the tamper switch 29 causes an interruption in power and the encryption/decryption module 28 stored in the volatile memory 23d to be erased. Without the encryption/decryption module 28, the encrypted data stored in the non-volatile memory 23c is useless.

In another embodiment shown in FIG. 4F, the game software 15 resides in an electrically erasable and programmable read only memory (EEPROM) 23e. If the cartridge housing 19 is opened, the tamper switch 29 closes and an erase signal from power source 27 causes the data stored in the EEPROM 23e to be erased. The practice of erasing data in an EEPROM is well known and need not be discussed in detail here.

It will also be appreciated that special enhanced security tournament cartridges 21 may be supplied to players for advanced rounds of competition in connection with any tournament.

Referring again to FIG. 4A, as a means of obtaining information as to where games are being played for compiling various tournament statistics and/or for preventing game play when the game computer 14 resides in certain locations, the game computer 14 may communicate with or have an integral Global Positioning System ("GPS") 37. A GPS receiver derives positional information from a plurality of satellites. The GPS information may be used to prevent game play in certain locations by providing a location lockout feature in the game software 15. When the player attempts to begin a game on the game computer 14, the game software 15 queries the GPS 37 and checks whether the current location of the game computer 14 is within an allowed area. This allowed area may be incorporated into the game software 15. If the game computer 14 is found to be outside of an allowed area, the game software 15 directs the game computer to deny player access to the game. In a

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different application, the positional information may be incorporated into the Authenticatable Outcome Message AOM and uploaded to the central computer 12 when a player submits his or her game outcome.   In this regard, the central computer 12 can use the positional information for
5        ranking/rating players without requiring submission of the player's specific location (i.e., the home address), and/or for compiling statistical location data.   The central computer 12 can ascertain which state, municipality or even town where the game computer 14 was located or, if the
10       player was mobile, all areas where the player was located when the player played the game, either by uploading the information from the game computer or by accessing a database containing such information.   Most GPS receivers have the capability to store a sizable amount of data.
15       Typical hand-held GPS receivers used in aviation applications can store enough information to save positional data for an entire flight.   Although current GPS satellites are subject to having their GPS signals degraded by the military without notice, future civilian systems that are
20       currently under development will be capable of providing consistently accurate positional information to within a few feet.

            To preclude player substitution, biometric identification devices such as a fingerprint reader, voice
25       recognition system, retinal scanner and the like, may be used to provide absolute player identity verification at the game computer 14. An example of such a device is the FC100 FINGERPRINT VERIFIER 31 available from Startek, a Taiwanese company. The FC100 is readily adaptable to any PC via an
30       interface card 39. The fingerprint verifier 31 utilizes an optical scanning lens. The player places his or her finger on the lens, and the resulting image is scanned, digitized, and the data compressed and stored in memory location 35. Typically, a 256 byte file is all that is required. Each
35       live scan fingerprint is compared against the previously

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enrolled/stored template. If the prints do not match, access to the system can be denied. This procedure may be implemented before the initiation of a tournament game, during the game in response to prompts from the game software 15 at some predetermined or random times, or

5      continuously by incorporating the scanning lens into a joystick on the game computer 14 such that the player is required to maintain his or her finger on the lens at all times during the game for continuous verification. The fingerprint data may also be registered and stored in the

10     central computer 12 (either in its compressed form or as hash value) in a player information database for player identity verification during various protocols, and/or used as a key as described below.

A voice verification system which utilizes a

15     person's "voice-print" may also be used to provide player identity verification at either or both the central computer 12 and the game computer 14. The process of obtaining a voice-print and subsequently using it to verify a person's identity is well-known in the art, and therefore need not be

20     described in detail herein. One of ordinary skill in the art may refer to SpeakEZ, Inc. for voice identification/verification technology. Specifically, speaker identification software is utilized to take a sample of the player's voice. This sample is stored in the central

25     computer 12 in the player information database. Each time the player calls the central computer 12, it prompts the player to speak his or her name into the telephone 18. The speaker identification software then directs the central computer 12 to check the player's current voice-print

30     against the voice-print stored in memory. If there is a match, the procedure continues. This is described in more detail below. The voice-print may also be stored in a database in the game computer 14, to verify the player's identity at that location prior to allowing game play

35     without the central computer 12. This is also described in

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more detail below.

Referring now to FIG. 5, there are shown several exemplary ways in which the game computer 14 can generate an Authenticatable Outcome Message AOM. At the conclusion of the game, an outcome (e.g., a score) is displayed. In one embodiment, the outcome may simply be embodied in a code generated using any secret algorithm. This algorithm is not readily ascertainable or known by the player. It resides in the game software 15 or in a separate encryption/decryption module 28, and in the central computer 12. Accordingly, when the player seeks to register an outcome of, for example, 1,000,000 points for game XYZ, the game computer 14 generates an Authenticatable Outcome Message AOM, for example, 21328585, with the secret algorithm. The central computer 12, the same game computer 14, or any other game computer 14 applies an inverse of the secret algorithm to the Authenticatable Outcome Message 21328585, or the same algorithm to the score of 1,000,000 points for that game, and if the results match, the authenticity of the outcome is verified. Thus, an outcome cannot be created or guessed without actually playing a game on a game computer 14 containing the secret algorithm. In a preferred embodiment, the encryption/decryption module 28 generates an Authenticatable Outcome Message incorporating the outcome (and any play-related data) using the SSCID as an encryption key. This encryption of the outcome (and play related data) with the SSCID enables authentication of the outcome with respect to the particular game software 15 or game computer 14. Alternatively, the SSCID is combined with the outcome and the combination is incorporated into an authenticatable message with a different key. In this regard, the encryption of the outcome and SSCID may utilize the biometric data scanned with the fingerprint verifier 31 or obtained from the voice print system as described above as a key. In this manner, the player's identity may be verified in the authentication process. While the secret game software or

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computer ID, SSCID, is not made known to the player, it is possible to generate a known serial number based upon the secret number. In this connection, after the player powers up the game computer 14 for the first time, it implements a registration process. The SSCID is encrypted by the encryption/decryption module 28 and displayed on screen. The player calls the central computer 12, and enters the SSCID, along with his or her name and/or PIN. The central computer 12 then decrypts the SSCID and associates it with the player's registration information. The central computer 12 then generates a unique random number RS which is tied to the SSCID. The player writes this number down, and can use the same to identify his or her game computer 14 when authentication is not required. The same procedure can also be used to generate known serial numbers for secret software numbers. Software can also be tied to hardware. A player can be forced to register his new software before he plays the first game. In this regard, the game computer 14 displays the SSCID in encrypted form. The player calls the central computer 12 prior to initiating play. The SSCID is added to the player information database 48, and is then used in the authentication process of any outcome as described herein. This ensures that the player can only submit an outcome for authentication/certification when using his or her game computer and/or game software 15. Use of another player's game software 15 and/or game computer 14 will cause the authentication process to fail.

In yet another implementation, an outcome may be represented by other data or symbols which are intelligible only to the central computer 12, but not to the player. For example, the score 5000 is represented by symbol data comprised of three green dots, four brown squares and two purple triangles. After communicating the score to the central computer 12, the player is required to send this data for confirmation of the outcome. The player is unable to determine whether this combination corresponds to the

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same score, a higher score or a lower score. But the central computer 12 is able to decipher these symbols to determine if, in fact, they represent the same outcome submitted for certification in accordance with some secret coding protocol. Alternatively, the player is not provided with an actual score. The score is secret, and is revealed to the player by the central computer only after it interprets the symbol data. This is similar to encrypting or encoding the outcome.

In the case of tournaments, the Authenticatable Outcome Message AOM may prove tournament validity, by including data representing that the outcome was the result of a valid tournament game. This data may constitute a subliminal message within the Authenticatable Outcome Message AOM. Alternatively, the Authenticatable Outcome Message AOM may include all or part of the Authenticatable Start Message ASTM for initiating tournament play for this purpose.

Authenticatable Outcome Messages AOMs may also contain statistical data for enabling the sanctioning authority to compile market research information. This data may be compressed by the game computer 14, and decompressed by the central computer 12.

The game software 15 may be adapted to instruct the game computer 14 to save game play up to a certain point in a game, and to resume play from that point at a subsequent time. In this regard, a "resume code" may be generated, which enables a player to pick up a game from where he left off. The game play to a specific point may be stored entirely in non-volatile memory. This would allow golf tournaments in which players could stop after a number of holes had been completed, picking up play at a later time or date. Alternatively, the game computer 14 may generate an Authenticatable Outcome Message AOM that represents the game outcome to this point. This allows for a first player to "hand off" the AOM to the next player who continues the

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game. Such an arrangement is analogous to a relay-race scenario where a player runs a certain distance and then hands off an object to the next runner. It also enables the same player to resume game play without having to store the large amount of data representing the game play to the point of termination. Since game programs generate games that are typically segregated into various levels, where the player advances from level to level as the game proceeds, this "code" may be used to instruct the game software 15 to continue from any given point. When the player selects a "quit" or "end game" option, if the player desires to continue the same game at a subsequent time, he inputs the Authenticatable Outcome Message AOM into the game computer 14. If it is authenticated, then game play proceeds from the prior termination point.

To prove integrity of the game software 15 through the outcome certification process (i.e., that it has not been tampered with), digital signature protocols may be utilized. In this regard, a digital signature algorithm with a private key is employed to "sign" a message. This message may be a hash value of the software generated with a function, a compressed value of the software code produced by a compression algorithm, and the like. The signed message is then verified using the digital signature algorithm with a public key at the central computer 12, the same game computer 14 or any other game computer 14 in the system. The secret key may reside in the encryption/decryption module 28, and preferably, the encryption/decryption module 28 resides in a secure perimeter 300 as discussed above. The secret key may be the SSCID, and/or a hash or compressed value of the digitized biometric fingerprint data or voice print described above. The public keys may be contained in the KDC at the central computer 12 as mentioned above, to enable players to verify the digital signature of the software at their respective game computers 14.

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In an exemplary embodiment, the encryption/decryption module 28 generates a hash value of the software instructions which make up all or part of (i.e., game program 26) the game software 15. This hash value is incorporated into the Authenticatable Outcome Message AOM. The hash value is generated using a one-way hash function which operates on a numerical representation of the game software 15. An example of a one-way hash function is the Secure Hash Algorithm ("SHA"). SHA is a U.S. government standard, and is specified in FIPS PUB 180 of the National Institute of Standards and Technology. Other examples of hash algorithms include MD4, MD5, RIPE-MD, Haval, etc. One skilled in the art may refer to *APPLIED CRYPTOGRAPHY*. As a specific example, each character of the game software 15 may be converted to ASCII values and then into a binary series of 1s and 0s. An exemplary one-way hash function may operate on this series as follows: (1) exchange the positions of all 1s and 0s; (2) group the digits into blocks of 64 digits each; (3) raise each block to the 5th power and then truncate the result to 64 digits; (4) take the final complete number and square it; (5) convert this binary number to base ten; and (6) take the last 24 digits as the hash value. The initial hash value for any given copy of the game software 15 is created prior to sale or distribution, and may be stored in the central computer 12, or even publicly known. This hash value may be derived from a different one-way hash function for each copy of game software 15 sold. If the player attempts to alter the game software 15 by tampering with the software instructions to produce a more favorable game outcome (i.e., a higher score or faster time to completion), such modifications to the game software 15 will be evidenced by the mismatch between the newly generated hash value and the initial hash value stored in the central computer 12.

Since the game software 15 may be tampered with while the software instructions reside in the volatile

read-write memory (i.e., RAM) of the game computer 14, tampering may not be detected by just generating a hash value of the entire game software 15 at the end of a game. One way to detect and provide evidence of tampering of the game software 15 while it resides in RAM, is to have the secure CPU 302 of the secure perimeter 300 periodically check blocks of the game software 15. In this connection, referring now to FIGS. 6A and 6B, the game software 15 may be configured with n blocks 314 of instructions, where each block 314 has an associated hash value $h_1...h_n$ determined by using a one-way hash function. Similarly, a master hash value $h_m$ of all the block hash values $h_1...h_n$ is also determined using a one-way hash function. These values may be stored in the secure memory (ROM 304 or other non-volatile memory 313) of the secure perimeter 300. The secure memory of the secure perimeter 300 may store such values for many different games. At step 316, a block is loaded into the RAM of the game computer 14, and its instructions are executed at step 318. When the block 314 of software instructions is to be replaced in the RAM of the game computer 14, that block 314 is read by and loaded into the secure RAM 308 of the secure perimeter 300 at step 320. The secure [perimeter] CPU 302 calculates a hash value $hsp_n$ of that block 314 using the one-way hash function at step 322, and the computed hash value $hsp_n$ is compared to the known hash value $h_n$ for that block 314 stored in the secure memory of the secure perimeter 300 at step 324. If the computed block hash value $hsp_n$ matches the expected value $h_n$, and the game is not over at step 328, the next block of instructions 314 that replaces the previous block, represented by incrementing n at step 330, is loaded into the RAM of the game computer 14. If a block hash value $hsp_n$ does not match $h_n$ at step 326, the secure CPU 302 can do several things. It can send a message to the game computer 14 to disable the game program 26 at step 328. Alternatively, the secure CPU 302 generate a tamper

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indication which is included at the end of the game in the Authenticatable Outcome Message AOM at step 332, or, if no tampering is detected, it can generate a non-tampering indication which is included in the Authenticatable Outcome Message AOM at step 329. Thus, when the player attempts to
5    submit an outcome for certification which was obtained with tampered game software 15 as evidenced by the tamper indication, the central computer 12 can reject the outcome. The secure CPU 302 in the secure perimeter 300 may alternatively calculate a master hash value $hsp_m$ based upon
10   all of the individual block hash values $h_1...hsp_n$ that were calculated as each block 314 was examined. This master hash value $hsp_m$ may then be compared to the expected master hash value hm stored in the ROM of the secure perimeter 300. If the master hash values do not match, the secure CPU 302 in
15   the secure perimeter 300 can generate a tampering indication which is incorporated into the Authenticatable Outcome Message AOM. Alternatively, the master hash value hspm itself may be incorporated into the Authenticatable Outcome Message AOM, and subsequently verified at the central
20   computer 12 as described above.

In the case of a dedicated game computer 14 (i.e., a game console), where it is more difficult to access and alter software instructions while loaded in RAM, or where the requisite level of security is not great, the secure
25   perimeter 300 may not be required. However, there exists a problem unique to game consoles in the form of the GAME GENIE video game enhancing device. The GAME GENIE is an interposing device that connects between a game cartridge 21 and the game computer 14. In a game console application,
30   the game software 15 resides in the ROM of a dedicated game cartridge 21. The interposing device enables a player to temporarily change certain game play-features by altering program instructions that are loaded from the ROM of the game cartridge 21 into the RAM of the game computer 14.
35   These changes are not permanent, and disappear when the

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power to the game computer 14 is turned off. This provides a unique challenge in the context of the present invention, where the certification aspects rely, in part, on verifying the integrity of the game software 15. The present invention overcomes the interposing device problem by utilizing one-way hash functions and encryption in authentication protocols.

As in the above example incorporating one-way hash functions, the game software contains n blocks 314 of software instructions, where each block 314 has an associated hash value $h_1 \ldots h_n$ and the entire set of instructions has a master hash value $h_m$ computed by applying the individual hash values $h_1 \ldots h_n$ to a one-way hash function. The hash value of each block may be determined with the same or a different one-way hash function. One of the blocks 314 may contain a list of all hash values for the other blocks, the master hash value $h_m$, and the hash function or functions used for calculating each block hash value and the master hash value. As described above, the master hash value $h_m$ may be stored in the game software instructions, or can be input by the player into the game computer 14 at the start of a game. Thus, the master hash value $h_m$ is checked at game start by initially computing the master hash value hnewm from the values $h_1 \ldots h_n$ to determine whether it matches the value which is either stored in the instructions or input by the player. If the interposing device was used to modify any of the instructions, the computed master hash value hnewm will not match the input master hash value at the game start. To ensure that the interposing device does not subsequently alter software instructions as they are loaded into RAM, the game software 15 contains instructions that direct the CPU 27 to compute the hash value $hnew_j$ of each block j as it is to be replaced in RAM, and a recalculated master hash value $hnew_m$ based upon the new hash value for block j and the known hash values of all the other blocks. The calculated master hash

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value $hnew_m$ is then compared to the known master hash value $h_m$ in the hash block. If at any time a discrepancy is found, the game software 15 may instruct the game computer 14 to disable the game software 15, or generate a tampering indication that is included in the Authenticatable Outcome

5      Message AOM. In this connection, the hash values $h_1 \ldots h_n$ and hm, the one-way hash functions, and the instructions for checking the game software instructions in this manner may reside in a ROM chip internally associated with the game computer 14. Thus, although the protocol is essentially the

10     same, no "security" instructions are executed from the game software 15 itself.

Another solution to the interposing device problem resides in the use of an authentication protocol to enable the game software to run in the game computer 14. In this

15     connection, the game software instructions stored in the ROM of the game cartridge may be made authenticatable where the game computer 14 authenticates the instructions prior to executing the program. This can be implemented by encrypting the software instructions with a private key by the game

20     developer, thereby requiring that the game computer 14 decrypt the encrypted game software instructions with the corresponding public key prior to execution of the game program. The instructions and algorithm(s) for performing the decryption process reside in a ROM chip (not shown) in

25     the game computer 14. The game software instructions are encrypted in blocks. Before each block is executed in the RAM of the game computer 14, it is decrypted by the CPU 27 with the algorithm(s) and keys stored in the ROM chip. If an interposing device is used to make changes to the game

30     software instructions, the authentication process implemented by decrypting the encrypted game software instructions with the public key will reveal unintelligible commands and the game program will be altered. This alteration may be detected by a security program in the ROM

35     chip and used to disable the game software 15 and/or

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incorporated into the Authenticatable Outcome Message AOM to indicate tampering.

In addition to cryptographic techniques for defeating GAME GENIE-type devices, there are other methods that can be equally effective. One technique is to authenticate not only the score of the game but several key characteristics of the game. A GAME GENIE, for example, might allow a player to be completely invulnerable to the attacks of opponents. If the game he or she were playing required, for example, defeating a dragon at the end, the GAME GENIE enhanced player would have no trouble quickly defeating the dragon. Most players would take a longer amount of time and would likely sustain more damage as a result. This information (e.g., number of seconds elapsed and units of damage sustained) could be included in the Authenticatable Outcome Message AOM so that the central computer 12 can compare it to known information to determine whether it was within "normal" bounds. If it was outside normal bounds, the central computer 12 may initiate a challenge/response protocol involving the game computer 14, including certain register values (such as invulnerability status) in the reply message. Rather than detecting the presence of a GAME GENIE directly, this protocol detects the end-effects of a GAME GENIE. Software obfuscation techniques can also be used to effectively hide how the game software 15 works, as is well known in the art. Reverse engineering obfuscated software requires considerable time, delaying the creation of GAME GENIE produced cheat codes. Since CD-ROMs must be re-mastered every ten thousand or so pressings, it is possible to create many different versions of the game software. Thus, a GAME GENIE device would have to generate cheat codes for every possible software variation.

Another solution is to monitor the time interval between the time the game computer 14 loads the game program 26 and the time that the game actually starts. If a GAME GENIE device were being used, the game would not start

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immediately since the player has to enter the cheat codes into the game computer 14 prior to game start.

In yet another embodiment, storing all the game data on removable memory media may enable the central authority to subsequently determine if the game was created with cheat codes input by a GAME GENIE device. This data may be "recorded" as described in detail herein.

The above described tampering indications may be incorporated into the Authenticatable Outcome Message AOM as "subliminal channels" of information, i.e., information which is difficult to decipher. In addition to the hash value and encryption authentication protocols described above, the game software 15 may run an integrity check on itself consisting of, for example, performing a one-way hash of the current memory registers to obtain a hash value. It then determines whether this hash value is within an allowable range of possible hash values stored as a line of code in the game program. If the determined hash value is within the allowable range, it returns a tamper indication value of 0 (i.e., no tampering made or attempted). If the determined hash value is outside the allowable range, it returns a tamper indication value of 1 (i.e., tampering made or attempted). This tamper indication value 0 or 1 is appended to the outcome and incorporated into the Authenticatable Outcome Message AOM. When the Authenticatable Outcome Message AOM is authenticated by the central computer 12, the tamper indication digit is interpreted to indicate whether that copy of the game software 15 has been altered or modified. These messages may be arranged so as to render them very difficult for a hacker to interpret their meaning. For example, in the string 13000087457, the last digit "7" is a pointer to the seventh digit in the string - "8", where the fact that this digit is an even number indicates that tampering was attempted. Similarly, the game software 15 may generate scores in specified multiples, e.g., five, such that any

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score not ending in a five or a zero is invalid. Furthermore, the game software 15 may vary one digit in the score to indicate tampering therewith in accordance with a self-integrity check as described above. For example, a score of 3905 is valid, but if the score is 3905 + 1 = 3906, the score is rejected because the addition of the numeral 1 indicates tampering.

The natural random variations in the magnetic memory media on which the game software 15 is made available, may be detected and used as a secret or private key in the cryptographic protocols described herein. These characteristics include variations in coercivity, granularity, coating thickness, surface profile, and the like. Thus, each specimen of memory media has a unique "memory media signature" that is dependent upon these characteristics. One method of detecting this unique memory media signature is disclosed in U.S. Patent No. 5,235,166 to Fernandez. The Fernandez Patent teaches detection of the relative position of specific features of signals derived from the output of a transducer, and then measuring the deviation between the precise location of peak points, known as "jitter." The jitter is dependent upon the media itself, and the associated data stored thereon. The jitter may be represented as a digital mapping, i.e., a table of all the values of the jitter, or a sample of the jitter; a checksum of quantities derived from a number of jitter quantities or a one-way function (to produce a hash value); or multiple checksum quantities corresponding to jitter in different regions of the media. An initial memory media signature may be stored in the central computer 12 in the tournament database 46 in memory area 80 (see FIG. 7) prior to sale or distribution of the game software 15. Thus, during the outcome registration process described below, this information may be read from the memory media such as the game cartridge 21, using hardware such as that disclosed in the Fernandez Patent. This information may be included in

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the Authenticatable Outcome Message such that the central
computer 12 can compare the initial memory media signature
to the current memory media signature at the time an outcome
is submitted to the central computer 12 for certification.
Alternatively, the memory media signature may be used as an

5      encryption key for authentication. In the case of the
former, if the newly computed memory media signature does
not match the initial memory media signature, the game
software 15 has been altered and/or an unauthorized copy has
been made. If the memory media signature is used as an

10     encryption key, the outcome can be authenticated with
respect to that copy of the game software 15. Another unique
key that may be utilized in the cryptographic protocols
herein is the unique identification number built into some
central processing units. Intel, for example, assigns a

15     unique number to each of its processors. This number may be
read by the encryption/decryption module 28 each time an
authenticatable message is to be generated. This unique
number may also be referred to as the SSCID in accordance
with the definition thereof as described above.

20          Referring now to FIG. 7, there is depicted an
exemplary memory arrangement for the central computer 12.
Here again, although the central computer 12 is shown as a
single unit, it may be comprised of a network of computers.
Specifically, the central computer 12 includes a memory 42

25     containing several relational databases. These include a
game database 44, tournament database 46, player information
database 48, outcome database 50 and a statistics database
51. The central computer memory 42 also includes an
encryption/decryption module 52, transmission error check

30     module 54, rating/ranking module 55, time/date module 56 and
an operating system 58. The transmission error check module
54 functions to detect inputting errors, corruption of
transmitted data due to communication problems such as line
noise and the like, to enable a resend command or request to

35     be made as described above with respect to the game computer

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14 and as is well known in the art. The operating system 58 controls the central processing unit 60 of the central computer 12. A clock 62 provides signals to the central processing unit 60 in a conventional manner and is also used by the time/date module 56 for time-stamping incoming messages as required. Alternatively, a trusted digital time stamping service may be used for this function.

The game database 44 includes game ID data in memory area 64, a game name in memory area 66 and tournament ID data in memory area 68.

The tournament database 46 includes a tournament ID number which uniquely identifies each tournament in memory area 70, the corresponding tournament date or range of dates over which the tournament is in effect in memory area 72, the division levels for a given tournament (tournaments may contain multiple levels such as beginner, intermediate and/or advanced), in memory area 74, information with respect to prizes to be paid out for a given tournament or fixed prizes for certain high scores for any game in memory area 76, the SSCIDs and software serial numbers SSNs in memory area 78, an initial memory media signature in memory area 80, an initial hash value of the game software 15 in memory area 82, entry fees paid or pre-paid for a given tournament in memory area 84, a start message for enabling a given tournament to begin in memory area 86, outcome messages received from the game computers 14 in memory area 88, and a list of qualification points earned to enable certain players to enter future tournaments in memory area 90.

A player follows a registration process whereby personal information such as his or her name, address, phone number, age, etc., is provided to the central computer 12 and stored in the player information database 48. The player may return a registration card included with the game software 15. This registration card contains the player's identification data and the serial number SSN of the game

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software 15. The registration card may contain security indicia such as a hologram and/or secret ID which is difficult to forge. This may allow the central authority in the case of game software 15 for use on a home PC or which resides in a game cartridge for use with a game console, to
5  ensure that the game software 15 is associated with a specified player. The player is assigned a player ID which is used to register for tournaments and in the outcome registration process, either in connection with a tournament or independent thereof. The player may also be required to
10  provide biometric data from the biometric device 31 (such as the digitized, compressed or hashed fingerprint data obtained from a fingerprint scanner, a voice-print obtained from a voice verification system, or the like). The player information database 48 includes the player ID and/or
15  biometric data in memory area 91, the SSCID in memory area 92, the player's name/address/phone number/age in memory area 94, team ID number for team affiliation in the case of team tournaments in memory area 96, a list of tournaments in which the player has competed in memory area 98, and
20  qualification points earned to enable the player to engage in future tournaments in memory area 100. Furthermore, memory area 101 stores player handicap values which may be used to adjust a player's outcome for a given tournament game or to modify the difficulty of a game generated by the
25  game software 15 by communicating the game difficulty level in an Authenticatable Start Message described below. Handicap values may change in accordance with that player's demonstrated skill level for a particular game. In a golf tournament, for example, handicap values based on prior
30  tournament results would be used for subsequent tournaments. Weak players may have strokes deducted from their final score while strong players had strokes added to their score. Instead of having strokes added or subtracted from the score, the handicap could also take the form of a set number
35  of "mulligans", which allow the player to take back a poor

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shot and try again. Player rankings, ratings and titles may be stored in memory area 103, as well as in the outcome database 50.

The outcome database 50 includes the tournament ID in memory area 102, a listing of outcomes (i.e., scores, times of completion, or other special characteristics of the game) for any certified or tournament game and the corresponding player rating/ranking/title in memory area 104, and the player's name or team affiliation(s) in memory area 106.

The statistics database 51 may include game ID data in memory area 51a, player and team data in memory area 51b, and various statistical information in memory area 51c. The statistics database may be accessed over the telephone, or through an on-line service. It may or may not be password protected.

Referring now to FIG. 8A, there is depicted a flow-chart of an exemplary tournament entry procedure in the present invention. For the purpose of illustration, the flow-chart refers to a system where the player manually or verbally (through voice responsive hardware/software) enters messages into the telephone 18 in response to prompts from an IVRU as shown in FIG. 1. However, it will be appreciated by persons skilled in the art that messages may be communicated between the game computer 14 itself and the central computer 12 by establishing a direct link or on-line connection as shown in FIGS. 2 and 3. In one embodiment, all games are tournament games. In another embodiment (shown in FIG. 8A), when a player activates the game computer 14 to play a game in the usual manner, the game software 15 directs the game computer 14 to generate an option to either enter a tournament or to play a regular game at step 108. If the player chooses to play a regular game, the game computer will generate a game in a conventional manner at step 110. If the player selects the option to engage in a tournament, the player may be required to proceed with a biometric

verification procedure at step 111, using for example, the fingerprint verifier 31 or a voice-print check. The game software 15 will enable the tournament options at step 112. These may include disabling cheat codes as well as other modifications to the game parameters in the game software 15. The game software 15 also directs the game computer to display a tournament ID for a given tournament, and a toll free 800# for the player to call at step 114. The use of an 800# is intended to be exemplary. Different tournaments may utilize 900#s which charge a prescribed toll fee, some or all of which may be applied to a tournament entry fee. In the case of 900#s, a blocking system to prevent children from calling may be utilized. This can be implemented by setting up a special blocking phone number operably connected to the central computer 12 that provides for blocking access from a given originating telephone number or by a person with a specific PIN. In the case of the former, the central computer 12 can deny (block) tournament entry requests for calls made from a specific telephone number that is identified in a database as "blocked." Identification of the originator of the 900# call may be made using an Automated Number Identification ("ANI") system of the type well known in the art. If the block is by PIN, the central computer 12 can identify blocked PINs by storing a list thereof in a PIN-blocking database. It is also anticipated that the 800# and tournament ID may be contained in separate literature accompanying the game software 15, and therefore need not be displayed by the game computer 14. However, for the purpose of illustration, the following description describes a system where the 800# is displayed by the game computer 14. The player then dials the 800# and connects to the central computer 12 via the IVRU 16 at step 116. The IVRU 16 prompts the player for the player's ID at step 118. The player enters his or her player ID into the keypad of the telephone 18 in a conventional manner at step 119. The player ID is communicated to the central computer

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14, which checks the player information database 48 to determine whether the player has a valid player ID at step 120. If not, the player registers with the central computer 14 at step 122 as described above. If the player ID is verified, the IVRU 16 prompts the player for the tournament ID, and the software serial number SSN for the game software 15 at step 124. The player enters the tournament ID and game software serial number SSN into the telephone 18 at step 126. Since a given tournament may have different divisions (e.g., beginner, intermediate and/or advanced), the player may have the option to choose the appropriate level of competition for his or her skill level. If the player has the option, the IVRU 16 prompts the player for the division number of the particular tournament at step 128. At step 130, the player then enters the division level or number into the telephone 18. Alternatively, if the player already has a rating stored in the outcome database 50, the division level may be determined by the central computer 12. The tournament database 46 maintains a record of the divisions for a given tournament in memory area 74. The central computer 12 may limit the number of entries in a tournament, and may thus verify that space is still available when a player seeks entry in that tournament. If space is available, the central computer 12 checks whether the player has pre-paid for tournament entries or whether a pre-paid number of tournament entries were included in the purchase of the game software 15 at step 132. It is anticipated that payment for tournament entries may also be made through an account arrangement where the player pays a certain fee on some prescribed basis. Entry fees for the tournaments are stored in memory area 84 in the central computer 12. If no entry fees have been paid, the IVRU 16 prompts the player for a credit card number at step 134 if an entry fee is required. The player enters the credit card number into the telephone at step 136. The credit card validity is checked on-line in a conventional manner. Of course, credit card use

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and authorization could be made by swiping the credit card through a credit card reader, in lieu of manually entering the credit card number into the telephone 18. In an alternative embodiment, entry in a tournament may neither require payment of any fees, nor a start message to enable
5      tournament play. At step 138, the central computer 12 then generates an Authenticatable Start Message ASTM for the player's tournament game and specific game software 15 using the encryption/decryption module 52. The Authenticatable Start Message ASTM may be encrypted so that only the
10     intended game computer and/or game software can use that message when decrypted with the required key. The Authenticatable Start Message ASTM is communicated to the player over the telephone 18, and then entered into the game computer 14 by the player at step 140 (e.g., through the
15     computer keyboard or a joystick). In this connection, the Authenticatable Start Message ASTM can enable tournament play based upon public sources of randomness. In such an implementation, the player is required to enter both the Authenticatable Start Message ASTM and a separate input
20     available from some public source such as television, radio, newspapers and the like. Thus, tournament play starts when players are able to obtain an initialization code from, for example, the lottery Pick "4" numbers broadcast on the 10 o'clock news on channel Z. This random information must be
25     entered prior to game play and becomes part of the Authenticatable Outcome Message AOM. Thus, this information verifies that the game started subsequent to the occurrence of the triggering event. The game computer utilizes the encryption/decryption module 28 to authenticate the
30     Authenticatable Start Message ASTM at step 142. The authenticated start message signals the game program 26 to enable play of the tournament game at step 144. The start message signals the game software 15 that this is a valid tournament game. It may also contain instructions for the
35     game software 15 to generate specified or random

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initialization parameters for that tournament game, such as, for example, its challenge level. In this connection, it is anticipated that start messages may be broadcast over mass communications channels such as television, radio, the Internet, etc., at a specified time to start a given tournament or to start player competition in a race of skill. That implementation will be described in more detail below.

The start messages may be used to facilitate game play on a pay-per-game basis. For example, a player calls the central computer 12, pays a specified fee, and obtains a start message good for one game or a number of games. Thus, a game computer 14 in a home environment can be made to operate much like an arcade machine. In this application, the start message functions as a key; without it, the game software 15 cannot generate a game. By incorporating a timing element into the start message, it can be made "time-limited" (i.e., valid for some predetermined period of time), assuming that the game computer 14 communicates with a clock, preferably one that is tamper-resistant. A variation of the pay-per game concept is described in another aspect of the invention in more detail hereinbelow.

The start messages may also be used for other applications. A start message may contain compressed advertising information, which is decompressed by and displayed on the game computer 14.

Referring now to FIG. 8B, in a coin-operated arcade environment, a player may purchase a number of tournament entries embodied in a pre-paid card 216. The card 216 may include memory media such as a magnetic strip. The game computer 14 in the case of an arcade machine, for example, has a card reader 218 communicating with the CPU 27 of the game computer via an interface in a conventional manner. The card 216 is obtained from a tournament operator station 220 having a card encoder 222 communicating with the

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central computer 12. The tournament operator station 220 may have a tournament operator or may itself consist of an automated apparatus which accepts payment and tournament entry requests. The player pays the tournament operator by credit card or cash. If payment is made by credit card, the

5    tournament operator obtains on-line authorization in a conventional manner, and then sends a tournament entry request to the central computer 12. If payment is made by cash, the tournament operator simply sends a tournament entry request to the central computer 12. Upon receipt of a

10   requested number of tournament entries, the central computer 12 generates a corresponding number of Authenticatable Start Messages ASTMs and communicates the same to the operator station 220 where the start messages may be encoded in the magnetic strip of card 216. The word "message" in this

15   context is used to identify each tournament entry instruction or code. It is anticipated that a single message may contain a plurality of entry codes for a corresponding number of tournaments. Thus, the reference here to Authenticatable Start Messages ASTMs means multiple

20   tournament entries. When the player swipes the card 216 through the card reader 218, the Authenticatable Start Message ASTM associated with that tournament is read by the game computer 14 and enables tournament play as described above.   The Authenticatable Start Message ASTM may be

25   customized to enable tournament play on a particular game computer 14 or its game software 15 that is identified (e.g., using the SSCID) in the Authenticatable Start Message ASTM.   In another application, the Authenticatable Start Message ASTM may contain unique identification information

30   to be incorporated into the Authenticatable Outcome Message AOM such that the right to submit an outcome represented by a given Authenticatable Outcome Message AOM can take place only once. Thus, a player cannot steal an Authenticatable Outcome Message AOM representing a high score obtained on

35   another game computer 14, because the central computer can

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determine that the Authenticatable Outcome Message AOM representing that specific score from a given game computer 14 was already used. To facilitate the application of the present invention to the arcade environment, an arcade machine (the game computer 14) may include a printer that

5    prints out the Authenticatable Outcome Message AOM to make it easier for the player to register an outcome where access to a telephone may be limited. By printing a receipt with the Authenticatable Outcome Message AOM, the player may access the system from a telephone at a convenient location,

10   and no hardware for an on-line connection between the arcade machine and the central computer is necessary.    Referring now to FIG. 9, a game sequence flow-chart is depicted.  In step 146, the game computer 14 generates a game in a conventional manner. If it is a tournament game as described

15   above, the game software 15 may disable cheat codes, alter the game such that the game play characteristics may be randomized in comparison to a non-tournament version, make the game easier or more difficult to play, and/or unlock hidden levels of challenge in comparison to non-tournament

20   versions of the game. At step 148, the game software 15 tracks the time to completion and special play-related information pertaining to that game such as number of lives lost, amount of time spent per level, number of hidden treasures found, etc. At step 150, the player finishes the

25   game and the game software 15 generates an outcome. In certain embodiments, the game computer 14 may then generate an option for the player to choose whether or not to submit the outcome for certification at step 152. If the player desires to have an authenticatable outcome generated for

30   submission to the central computer (in the case of a tournament), or to subsequently prove to friends that a certain score was achieved, the Authenticatable Outcome Message AOM (or Authenticatable Timing Message ATM for races of skill) discussed above is generated at step 154. If not,

35   the player may return to play another game at step 156. In

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the case of a tournament, the player may not be provided with the option of whether to submit an outcome for certification.

Referring now to FIG. 10A, there is depicted a flow-chart of an outcome certification sequence in a tournament embodiment. In this example, the outcomes are submitted to the central computer 12. However, a similar procedure may be employed for just certifying outcomes independent of any tournament. In that case, the central computer 12 generates an updatable database of player scores and statistics, which may be accessed by players through an on-line service, over the telephone or the like. Statistics could include lists of past prize winners, or lists of the top players in the current tournament. To initiate the outcome submission process for a tournament game, the game software 15 directs the game computer 14 to display the 800# for the player to call at step 158 as described above. The player is prompted by the IVRU 16 to enter the tournament ID at step 160, which the player enters into the telephone 18 at step 162. The central computer 12 then accesses the tournament database 46 for the particular tournament identified by the tournament ID at step 164. The IVRU 16 then prompts the player for his or her player ID, software serial number SSN, and the Authenticatable Outcome Message AOM at step 166. The player enters the player ID, software serial number SSN and Authenticatable Outcome Message AOM into the telephone 18 at step 168. This step may include entering multiple Authenticatable Outcome Messages AOMs, or a single Authenticatable Outcome Message AOM may represent several outcomes. The central computer 12 may request a biometric verification of the player's identity using, for example, the fingerprint verifier 31 at step 169. The biometric verification procedure using the fingerprint verifier 31 is depicted in a flow chart in FIG. 10B. In step 169a, the fingerprint verifier 31 does a live scan of the player's fingerprint, which is digitized at step 169b, and

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the digitized data is then compressed or hashed by the game software 15 at step 169c. The compressed or hashed data is communicated to the central computer 12, which compares the current data to that stored in memory in the player information database 48 in memory area 91 at step 169d. If

5    the compressed or hashed data from the current scan of the player's fingerprint matches that stored in the player information database 48, the player's identity is verified and the outcome submission procedure continues to step 170. If not, the outcome submission procedure terminates. The

10   same biometric player identity verification procedure may be implemented with a voice print verification at the central computer 12.   The central computer 12 authenticates the Authenticatable Outcome Message AOM using the encryption/decryption module 52 at step 170, using any one

15   of the protocols described above.   Authentication ensures either identity and/or integrity, depending on the specific cryptographic protocols selected. The use of keyless hashing of the outcome, for example, would ensure outcome integrity, while encryption of the outcome with a sender's private key

20   would ensure identity of the player submitting that outcome. A digital signature (a hash encrypted with the sender's private key) would ensure both integrity of the outcome and identity of the sender. The central computer 12 may time stamp the message, or it may communicate with a trusted

25   third party such as a digital time stamping service to perform this function. Digital time stamping is known in the art and details thereof may be found in U.S. Patent Nos. 5,136,646 and 5,136,647. The Authenticated Outcome Message AOM is stored in memory area 88 of the tournament database

30   46. If the outcome is not authentic at step 174, it is rejected at step 176. If the outcome is authentic, the central computer proceeds to step 177. The central computer may check the integrity of the game software as described above at step 179, or proceed directly to step 178. If the

35   software integrity check at step 179 reveals tampering at

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step 180, the outcome is rejected. After the outcome is authenticated and the integrity of the game software 15 verified, the central computer 12 adds the certified outcome to the outcome database 50 in memory area 104 at step 178. In the illustrative embodiment, the certified outcome is referenced by the corresponding tournament ID in memory area 102, and the player's name in memory area 106. At step 181, the central computer 12 may generate an Authenticated Outcome Confirmation Message AOCM which, when communicated to the game computer 14, can be used by the game software 15 to cause the game computer 14 to display a certified scoreboard with language to the effect that a particular outcome (e.g., score) was certified by the central computer 12. The same Authenticated Outcome Confirmation Message AOCM can be utilized by the game software 15 to direct the game computer 14 to generate special symbols or medallions as a result of an established level of competence. These may be made to appear on the screen with an identification of the player during subsequent game play. In view of the competitive nature of video games, this feature greatly enhances their play value games by providing for recognition of the player's achievements.

At step 182, the central computer 12 generates a current player ranking in the tournament utilizing the rating/ranking module. The player rankings in the outcome database 50 may include subsidiary rankings delineated by state, school, age group, etc. If the tournament is still ongoing, players may be provided with their current ranking so far. The tournament ends at some predetermined time at step 183. At step 184, after the conclusion of the tournament, the central computer 12 sorts through all the scores to determine a winner from the final rankings. These may be segregated by division level such that the beginner, intermediate and advanced levels each have a separate winner with associated rankings and ratings. At step 185, prizes are awarded in a conventional manner.

Prizes or awards can also be offered on an exclusive basis to players who achieve certain skill levels. For example, becoming a "Two Star" SONIC THE HEDGEHOG player may require the certification of two scores of 50,000 points or more. Players reaching this level of achievement may be

5   offered prizes, or the ability to purchase goods at self-liquidating prices. There may be a catalog for "Two Star" players and a separate catalog for "Three Star" players. An award for achieving a certified level of play may include the incorporation into the game software if the

10  player's name in background graffiti on the actual game screens, renaming game characters, or the modification of the game play or visuals in some other subtle manner until a new higher scoring player is allowed to change these altered characteristics. The player information database 48

15  is also updated to reflect the tournament played and any qualification points earned towards future tournaments in memory area 100.

It is also anticipated that "instant prizes" could be awarded to players based upon exceeding some

20  predetermined threshold level (e.g., a player scoring over a million points for any MORTAL KOMBAT game receives a $50 instant prize). This prize could be implemented, for example, in the form of a $50 credit to the player's credit card. In a golf game embodiment, an instant prize can be

25  offered for a hole-in-one on each hole, supplementing a normal tournament prize structure for the lowest score. Alternatively, if the tournament is being conducted in an arcade environment, the player could receive an instant cash prize from an agent at the arcade. These instant prizes

30  could be awarded mutually exclusive and prior to the conclusion of a given tournament. The arcade is then reimbursed for all or part of the prize awards by the central authority. Other awards may include coupons good for discounted or free entries in future tournaments, frequent

35  flyer miles or hotel points, and/or for special cheat codes

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(which cannot be used, however, in connection with a certified game).

In accordance with another aspect of the invention, the game software 15 may contain a certificate printing routine which contains templates for generating

5   different certificates for players who reach a certain score and/or who attain a certain title (e.g., "Master," "Grandmaster," etc.). Such certificates may be printed out by the game computer 14, upon reading an Authenticated Outcome Confirmation Message AOCM from the central computer

10  12, and represent that a player has attained a given level of achievement. The Authenticated Outcome Confirmation Message AOCM "unlocks" the printing instructions in the game software 15. The player enters his or her name and/or password into the game computer 14. These certificates may

15  be customized for each game and even for different levels of achievement. A player bragging about his or her certified score to his or her friends may now proffer visual proof. A certificate could offer tangible evidence of a hole in one, for example. To prevent fraud, these certificates may be

20  provided with certain security indicia such as holograms and the like. If an unauthorized copy of the certificate is made, the security indicia becomes visible and thereby indicates that it is counterfeit. The certificate may also contain the Authenticatable Outcome Message AOM and/or

25  Authenticated Outcome Confirmation Message AOCM printed on the face thereof so that the achievement can be verified by calling the central computer 12.

The Authenticated Outcome Confirmation Message AOCM may also be utilized to unlock certain attributes of a

30  game that are only encountered by high scoring players. For example, a top scoring player who receives a certified score, may be provided with an Authenticated Outcome Confirmation Message AOCM that, when read by any game computer 14 with the game software 15, allows players on

35  that game computer 14 to view special hidden characters or

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final stages of the game, which are not normally encountered until a certain level or score is attained. In this regard, such an Authenticated Outcome Confirmation Message AOCM may include specific game software identification information such as the software serial number SSN to restrict its use to a specified game computer or number of game computers. Thus, the high scoring player provides the identity of those players and/or their game software serial numbers SSNs to the central computer, which incorporates the same into the Authenticated Outcome Confirmation Message AOCM. In this manner, the Authenticated Outcome Confirmation Message AOCM enables only those game computers 14 having game software 15 identified by those software serial numbers SSNs to reveal the hidden characters or final stages of the game. It is also anticipated that the central computer 12 may be utilized only to certify outcomes. Thus, a third party may be provided with the Authenticated Outcome Confirmation Message AOCM representing a certified outcome, where that third party then handles tournament rankings, ratings and prize distribution. For convenience, however, all such functions are schematically shown to take place with one central computer 12.

In all tournament embodiments, a final playoff protocol may be established for top players, which takes place in a central location under the supervision of a tournament director(s). Thus, where prizes for large sums of money are at stake, this final playoff may be used to ensure that no undetected substitution of players takes place during play or when reporting game outcomes.

Referring now to FIG. 11, an embodiment of the invention utilizing a challenge/response protocol is depicted. Steps 158-174 are identical to those shown in FIG. 10A and described above, and therefore need not be repeated. At step 186, the central computer 12 checks the tournament database 46 to determine whether the same Authenticatable Outcome Message AOM has been previously received, by

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checking memory area 88. If the Authenticatable Outcome
Message AOM presently communicated in this outcome
submission sequence is not the same as a previous
Authenticatable Outcome Message AOM, the central computer 12
generates an Authenticatable Challenge Message ACM for that
5 player's game computer 14 representing a key $k_x$ at step 190.
This representation may be the actual key $k_x$ itself, or an
instruction for the encryption/decryption module 28 to
retrieve the specific key $k_x$ based upon a certain command
(i.e., the command encrypt X means use encryption key $k_x$).
10 The Authenticatable Challenge Message ACM is communicated to
the player and entered into the game computer 14 at step
192. The encryption/decryption module 28 authenticates the
Authenticatable Challenge Message ACM and generates an
Authenticatable Response Message ARM based upon the outcome
15 with key $k_x$ at step 194. The player enters the
Authenticatable Response Message ARM into the telephone 18
at step 196. The Authenticatable Response Message ARM is
communicated to the central computer 12 and authenticated by
the encryption/decryption module 52 at step 198. If the
20 outcome represented in the Authenticatable Response Message
ARM is not authentic (where a player has stolen another
player's AOM), the outcome is rejected. A player cannot make
up an outcome because he cannot guess the key $k_x$ in advance.
If the outcome is accepted, the process proceeds in
25 accordance with the sequence shown in FIG. 9A. Referring
back to step 188, if the central computer detects a
duplicate Authenticatable Outcome Message AOM (i.e.,
indicating that a player may have stolen a message), it
generates an Authenticatable Challenge Message ACM
30 representing key $k_y$ (a different key than $k_x$ that was used to
challenge the earlier Authenticatable Outcome Message AOM)
at step 190'. The Authenticatable Challenge Message ACM is
communicated to the player and entered into the game
computer 12 at step 192'. The encryption/decryption module
35 28 authenticates the Authenticatable Challenge Message ACM

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and generates an Authenticatable Response Message ARM based upon the outcome with key $k_y$ at step 194'. The player enters the Authenticatable Response Message ARM into the telephone 18 at step 196'. The Authenticatable Response Message ARM is communicated to the central computer 12 and authenticated by the encryption/decryption module 52 at step 198. The rest of the process proceeds as described above. This protocol prevents someone from using another player's Authenticatable Outcome Message AOM to register a false score unless they also have access to the game computer 14 which generated the AOM.

In another embodiment, the challenge/response protocol utilizes random numbers in lieu of multiple cryptographic keys. In this implementation, the Authenticatable Outcome Message AOM includes a random number R1 generated by the encryption/decryption module 28. This makes each Authenticatable Outcome Message AOM unique, irrespective of identical outcomes on different game computers 14. If duplicate Authenticatable Outcome Messages AOMs are detected, the last Authenticatable Outcome Message AOM which is identical to a previous submission is rejected by the central computer 12. Non-duplicate Authenticatable Outcome Messages AOMs are processed as described above, to ensure that the player submitting the same is in possession of the actual game software 15 and/or game computer 14. This prevents· a player from stealing another's Authenticatable Outcome Message AOM and claiming it as their own. In this protocol, the central computer 12 generates a second random number RA2, which is included in the Authenticatable Challenge Message ACM. The ACM is authenticated by the encryption/decryption module 28 associated with the game computer 14, and then concatenated with the original outcome message into the Authenticatable Response Message ARM. The Authenticatable Response Message ARM is communicated to the central computer 12 and authenticated. The central computer 12 then checks whether

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R1 and R2 are identical. If they are, then the outcome message is verified as having been generated by the player's game software 15/game computer 14.

The challenge response protocol may be implemented by the central computer 12 on a random basis, or based upon current scores, previous scores, or some other game related factors, to streamline the score submission process. For example, every time an Authenticatable Outcome Message AOM is submitted to the central computer 12, it may generate a random number between 0 and n. If the random number is less than j (a number between 0 and n), the challenge/response protocol is initiated. If the random number is greater than or equal to j, the Authenticatable Outcome Message AOM is simply processed as depicted in FIGS. 7-11. This procedure saves time, as the player is only required to enter one message per outcome submission. And, if cheating is detected, it can be used to deny the player certification of future outcomes, and to invalidate any outcomes certified in the past as a penalty.

In another protocol, the Authenticatable Start Message ASTM as described above and illustrated in the tournament entry flow chart of FIG. 8A, includes a unique key for generating the Authenticatable Outcome Message AOM. Thus, every Authenticatable Outcome Message AOM is unique, regardless of whether multiple players have the exact same scores. Since every player is provided with a different key, the same outcome from different players would necessarily have to be represented by different Authenticatable Outcome Messages AOMs. This prevents a second player from stealing the Authenticatable Outcome Message AOM of a first player in an attempt to submit a false score. This is assured because the key sent with each Authenticatable Start Message ASTM is different, and the key for authenticating the message is different as well. If player X steals the Authenticatable Outcome Message AOM from player Y, player X will be caught cheating when the central

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computer 12 applies player X's key to the Authenticatable Outcome Message AOM that was encrypted with player Y's key (i.e., the message will be unintelligible). The Authenticatable Outcome Message AOM may also include the SSCID as discussed above to enable the central computer 12 to authenticate the message with respect to the specific game software/game computer as well.

Referring now to FIG. 12, in an embodiment for tournament races of skill, the above-described protocols may be used. There are two kinds of races of skill, one where all players start at a designated time, and another where players start at different times with the game software 15 time-stamping or tracking the elapsed time to completion for a given game. Where all players start at a designated time, a start message BSTM for a particular tournament may be broadcast over a mass communication channel such as radio, television or the Internet at step 204. There may have already been a registration process for the tournament as generally depicted in FIG. 8A and described above. The start message BSTM is entered into the game computer 14 at step 206, which enables the game software 15 to make a race of skill available for play. The game is started at step 208, and the time/date module 33 is initiated, using signals from the clock 36. The time/date module 33 tracks the elapsed time during the race of skill played at step 210. The time/date module 33 may accept signals from a secure clock that resides within a token such as the aforementioned iPower card or TOUCH MEMORY device. When the player has completed the race of skill, the time/date module 33 calculates the total time elapsed at step 212. At step 214, the encryption/decryption module 28 generates an Authenticatable Timing Message ATM (analogous to the AOM) representing the elapsed time. The Authenticatable Timing Message ATM is communicated to the central computer 12, which then authenticates the Authenticatable Timing Message ATM. For added security, the central computer 12 may

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time-stamp the ATM and any play-related data with the time/date module 56. If the player's time to completion is certified, a ranking, rating, title is then generated at the central computer 12 using any of the above-described processes. In an embodiment where races of skill start at variable times for a given tournament, the central computer 12 does not have to time-stamp the Authenticatable Timing Message ATM when it is received. The start message BSTM is used to enable a particular tournament, but does not start the time running for the competition (there may still exist a fixed period of time over which the tournament will be in effect). Instead, the time/date module 33, using signals from the clock 36 (which preferably resides within a secure perimeter), tracks the elapsed time during the race of skill from the time that the player begins the game (at his or her leisure) as described above, and the authenticatable time message ATM itself represents the time to completion.

Referring now to FIG. 13, there is depicted a flow-chart of an embodiment where players compete in a head-to-head tournament. This may occur in several ways. For illustration, the first player is identified as player "A" and the second as player "B." These players may compete against each other on a single game computer 14, or via an on-line connection between their respective game computers 14. If they play on the same game computer 14, the outcome of the game is simply incorporated into an Authenticatable Outcome Message AOM as described above. The player's respective PIN numbers may be included for identification purposes.

In an on-line head-to-head embodiment, at step 216, player A calls the 800# and connects to the central computer 12. The IVRU 16 provides an option for a head-to-head tournament game at step 218. Player A is prompted for his or her player ID at step 220, which is entered into the telephone 18 at step 222. Step 224 represents payment of an entry fee or verification of a

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pre-paid entry fee as described above with regard to the other embodiments. After fee payment is verified, the central computer 12 searches for another player at step 226, player B, who has requested entry in the same head-to-head tournament by completing steps 216-224. The central computer 12 then generates an Authenticatable Start Message ASTM based in part upon the player IDs for player A and player B at step 228. The Authenticatable Start Message ASTM is communicated to players A and B, and entered into their respective game computers 14 at step 230. One of the players then establishes an on-line connection to the other at step 232, and each game computer 14 verifies that the proper Authenticatable Start Message ASTM was entered at step 234. At step 236, players A and B proceed to play the game in accordance with conventional practice. It is known in the art to play many computer games in a head-to-head manner via an on-line connection. When the game is over, the game computer 14 of the winning player generates an Authenticatable Outcome Message AOM representing the outcome and the player IDs for players A and B, and the game software integrity information at step 238. The outcome submission process operates in essentially the same manner as described above, but includes player win and loss data to enable players to continue to compete in further elimination rounds.

An entire tournament for a group of players may be held on a single game computer 14. In this connection, the game software 15 may have the capability to set up a tournament schedule for multiple head-to-head matches. Players purchase machine readable codes or messages that, when entered into the game computer 14, are employed by the game software 15 to direct the game computer 14 to set up the tournament. The tournament format may be "round robin," where each player plays everyone else in the group, a "Swiss system," where a limited number of rounds are established with the players having the best scores being matched

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against each other (i.e., an elimination protocol), or some other format well known in the art. All players competing in the tournament enter their name and player ID into the game computer 14. The game software 15 generates the tournament schedule, and after each head-to-head match, records the outcome. At the conclusion of the tournament, a winner is declared and the tournament standings are printed on the computer display. The final outcome of the tournament may be certified by the central computer 12 utilizing any of the above-described protocols. Alternatively, each head-to-head game outcome may be certified by the central computer 12 in the same fashion.

Computation of player ratings is implemented by the rating/ranking module 55 in the central computer 12 using known principles. Alternatively, ratings may be calculated on the player's game computer 14. These ratings are dependent upon past player and opponent performance and skill. For example, player "A" may have achieved 5 wins and 5 losses against relatively weak competitors, while player B has 3 wins and 20 losses against world-class competitors. This makes comparison between players difficult. The player's respective ratings take the relative skill of the competitors into account. Chess ratings are a good example. In accordance with well-known rating protocols, such as those developed by the statistician Dr. Arpad Elo, chess ratings range from 0 to 3000 with a mean of 1500. Every 200 points represent one standard deviation from the mean. Thus, a rating of 2100 represents three standard deviations above the mean. The larger the rating differential between the stronger player and the weaker player, the greater the probability of the stronger player winning the match. A player rated 200 points higher than another player, for example, may be expected to win 75 games out of 100, while a player rated 400 points higher than another may be expected to win 90 games out of 100. After each game, points are added to the winner's rating and subtracted from the

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loser's rating. The number of points won or lost is dependent upon the rating differential. Therefore, defeating a "weaker" player in lieu of a "better" player causes relatively fewer points to be added to the winner's rating. The present invention provides for generating ratings for players of computer games. The player's new rating is calculated after the outcome of the head-to-head game is certified. An exemplary rating formula may be characterized as follows: Winner's new rating = old rating + ( x * rating difference) + y. If, for example, x - .04 and y = 16, and assuming a 2000 player beats a 1700 player, the 2000 player's new rating is computed as 2000 + (04 *(- 300)) + 16 = 2004. The loser's rating becomes 1696. If a 1300 player beats a 1500 player, the 1300 player's new rating becomes 1300 + (.04 * 200) + 16 = 1324. The loser's new rating becomes 1476. Thus, the greater the rating differential between players, the larger the rating changes after the games if the underdog wins. If the stronger player wins, his or her rating increases by a relatively smaller value. After new ratings are computed, the rating/ranking module directs the central computer 12 to update the player information database 48 and/or outcome database 50 to reflect the changes.

In any head-to-head embodiment, it is possible to equalize the playing conditions for players of differing ability by utilizing player handicaps to generate game initialization variables that provide the lesser rated player of the game with more lives, more ammunition and the like, or conversely, which reduce the number of lives, ammunition and the like for the higher rated player. These initialization variables may be included in the start message as discussed above for tournament games. Alternatively, they may be obtained from the game computer 14 itself or a separate handicap device 217 (i.e., preferably a hand-held computer comprising a central processing unit, memory, display and power source). The

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handicap device 217 may store various titles corresponding to different levels of achievement in its memory. A MORTAL KOMBAT game may have players classified as one star, two star, three star and the like. If player "A" is a two star player and player "B" is a four star player, the handicap device 217 calculates an equivalent rating for each player based upon these player's classifications. A two star player may have an equivalent rating of 1200 while a four star player may have an equivalent rating of 1700. Using these values, the handicap device calculates a rating differential of 500 points (1700 - 1200), and queries a database for that game that contains various handicap values and their rating point equivalents. Where a 1900 player competes with a 1600 player, the 300 rating point differential, for example, may disable the ability of the stronger 1900 player to "throw his or her opponent" (the weaker 1600 player). Doubling the amount of damage produced by "kicks" from the weaker player may be equivalent to a 500 rating point differential (e.g., the stronger player is handicapped by allowing the weaker player to double the damage and associated score with each kick). The handicap device 217 generates and displays a machine readable code compatible with the game software 15 that directs the game program 26 to set up the game with non-symmetrically altered characteristics. The methodology for altering game programs with manually input codes is well known in the art. The GAME GENIE device discussed above, and the use of cheat codes are good examples. In the case of the present invention, however, a similar protocol may be used in a novel application to equalize playing conditions by non-symmetrically varying the game in accordance with the differential between player ability. It is also anticipated that codes for handicapping the games may be obtained from printed materials that accompany the game software 15. These materials may have charts with rating ranges, handicaps and their corresponding codes. Alternatively, the game software 15 may include a handicap program that functions in the same

manner as the handicap device 217. In that case, the players simply enter their respective levels or even ratings into the game computer 14, and the game software 15 directs the game computer 14 to automatically equalize the playing conditions in the game pursuant to the associated player handicaps. Another way to equalize players of differing skill levels is to reduce the duration of the competition. Over the course of a dozen fights, the better player will almost always emerge victorious over the weaker player. Although he may lose a few fights, the better player will win the majority. If the competition is restricted to just one fight, the weaker player has a greater chance of winning.

Players may also be rated for games which are played not head-to-head, but against the game computer 14. In this regard, each computer opponent in the game software 15 is provided with a base-line rating. In MORTAL KOMBAT, for example, the computer opponent for the first level fight is relatively weak. As players win fights and proceed to higher levels, the opponents become increasingly powerful. Ratings can be assigned to each of these computer opponents. The first level opponent might be assigned a rating of 900, the second 1050 and the third 1300. These ratings may be determined in several different ways. In one embodiment, the computer opponents within the game software 15 are provided with arbitrary ratings. Players having known ratings from previous head-to-head competitions then play against these computer opponents. As a result of these matches, the ratings of the computer opponents within the game software 15 change in accordance with the rating formulas described above. The greater the number of matches played, the more accurate the ratings for the computer opponents. The player's Authenticatable Outcome Message AOM generated at the end of a match identifies which levels of the game were played and which opponents were defeated. Thus, the player's rating may be accurately calculated at the central computer

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12 by taking the play related data into account. If, using the hypothetical software rating numbers in the above-identified example, the player's Authenticatable Outcome Message AOM contains information that the player managed to defeat the first two opponents, but lost to the third, the player's new rating is calculated taking into account the player's previous rating, and result against the game software's rated opponents. This is analogous to defeating a 900 rated player, defeating a 1050 player, and losing to a 1300 player. This procedure may also be implemented for games that do not have such quantifiable levels. Ratings can also be assigned to overall measures of difficulty. In DONKEY KONG COUNTRY, for example, the game may have three difficulty levels such as beginner, intermediate and advanced. Finishing the game at the beginner level may add five (5) points to the player's rating, while completing the game at the intermediate level may add twenty-five (25) points to the player's rating.

In any of the above-described embodiments, players may register for tournaments in teams. These teams are identified by team IDs stored in memory area 96 of the player information database 48. The certified score (or time of completion for races of skill) for a particular team in any given tournament is an aggregation of the certified scores for all of the team members. Team standings, ratings and rankings are stored in the outcome database 50. Prizes may be awarded to members of the winning team. Players may also register as teams for league play, which is analogous to participation in a tournament.

While the foregoing descriptions refer to tournaments, the same protocol may be used to simply rank scores and/or to provide ratings for particular games. As with tournaments, the game software 15 may disable the cheat codes and scramble or randomize the predictable chain of events built in to the conventional program for a given game. The player calls the 800# listed to submit a

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particular score, and follows the sequence illustrated in FIG. 9, for example, except where the tournament ID is now just a game identifier. Thus, the player would not have to register in advance for a particular tournament. The player provides a game identifier and the Authenticatable Outcome
5    Message AOM to enable the central computer 12 to certify the score and generate a player standing, rating and ranking. In a simpler embodiment, players may provide scores for a particular game to the central computer 12 anonymously, such that the central computer 12 just compiles a standing of the
10   top X scores for the game. Players can then call in to obtain the top scores achieved to date for that game.

The foregoing protocols may also be employed to make predictions on future events, or for competitions such as fantasy baseball or fantasy football. For example, the
15   game software 15 generates a schedule of all or part of the football games for the upcoming weekend or even the season. The player makes predictions as to which teams will win, including any point-spreads which are determined by the game software 15, manually entered into the game computer 14 by
20   the player, or received from an external source, such as via an RF signal. The player's prediction may be converted to a hash value with a hash function, signed with the player's private key for authentication, and then encrypted with the central computer's public key to generate an Authenticatable
25   Outcome Message AOM. The Authenticatable Outcome Message AOM is authenticated and time-stamped by the central computer 12 or by the game computer 14 with a secure clock 36. The encryption of the player's predictions enables an authentication of the predictions. The time-stamp by the
30   central computer proves that the message was actually received at a particular time. After the events have transpired, the player provides the central computer with the actual predictions, which are compared to the actual outcomes of all the games. The central computer then
35   recalculates the hash value of the player's predictions to

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verify the same. Each player's performance may be compared to that of other players in a tournament scenario (i.e., player A's predictions are 85% accurate, while player B's predictions are only 60% accurate).

5   The protocols described herein may be used to select wagering elements for future games of chance of which the outcome is uncertain such as a Lotto game. In this connection, the game computer may generate at least one set of Lotto choices for a given Lotto drawing. The player's selections are incorporated into an Authenticatable Outcome

10   Message AOM which is time-stamped by a secure tamper-resistant clock, for example by the clock in an iPower card. In this manner, the central authority may authenticate the player's selections and verify that the player made those selections prior to the Lotto drawing.

15   Thus, the player need not "register" or submit his or her choices prior to a drawing.

The authentication protocols described herein may be readily adapted to games of chance including blackjack, craps, roulette, slots and the like. The use of

20   cryptographic protocols to evidence tampering with game software prevents a player from cheating the system or repudiating play. A blackjack player, for example, might pay the central authority a given fee for blackjack software that, when executed on the game computer 14, enables the

25   player to play a number of potential hands. The final outcome of this play is incorporated into an Authenticatable Outcome Message AOM that is submitted to the central computer 12. If the central computer 12 certifies the outcome, the player is paid any winnings, either directly

30   or, if a wagering account exists a credit may be made thereto.

The foregoing description as applied to game outcomes is equally adapted to tests taken on a testing computer 14. A test outcome may be authenticated as having

35   been achieved on that testing computer 14 by following the

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same protocols. Generating the test questions on the game computer 14 is analogous to generating a game, and the test taker's answers to those questions (i.e., the outcome) is analogous to a game outcome. In addition, tests can be presented with their questions in a random fashion.

5 Similarly, test takers may be ranked and rated by their scores in the same fashion.

Referring again to FIG. 1B, there is depicted a schematic and flow-chart of another embodiment of the invention wherein each game computer 14 is capable of

10 self-authenticating its own outcome. In this connection, the game computer 14 may be capable of printing an outcome accompanied by the words "certified." What this means is that the outcome of that game is capable of being verified as accurately reported and fairly achieved by the game

15 computer 14 itself. For example, after the conclusion of the game, the game computer 14 generates the Authenticatable Outcome Message AOM that constitutes the outcome of the game and a software tamper indication such as the digital signature of the game software as described above. The

20 private or secret key, such as the SSCID, used for generating the Authenticatable Outcome Message AOM provides for uniquely associating the outcome with that game computer 14. It also enables the Authenticatable Outcome Message AOM to be subsequently verified by authenticating the AOM with

25 the public key of a private key/public key pair, associated with the encryption/decryption module 28. If the tamper indicator such as the software hash value is publicly known, for example placed on the outside of the game cartridge (whether or not the hash function is secret), then when the

30 Authenticatable Outcome Message AOM is authenticated, the integrity of the game software 15 can be verified by the digital signature. For example, if the hash value incorporated into the Authenticatable Outcome Message AOM matches the known hash value for that game software 15,

35 there has been no tampering with the game software 15 and

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the associated outcome is certified as having been accurately reported and fairly achieved. Thus, a player who seeks to prove that his or her score is indeed certified, need only enter the Authenticatable Outcome Message AOM into the game computer 14, which reads and authenticates the Authenticatable Outcome Message AOM in accordance with the protocols described above with regard to the central computer 12.

The system also allows for mutual-authentication for an outcome from one game computer 14 to take place on any other game computer 14. As described above, if the Authenticatable Outcome Message AOM was generated with a secret or private key, a player seeking to authenticate that outcome on another game computer 14 must obtain the public key associated with the public key/private key pair. In this regard, a list of public keys may be stored on a database at the central computer 12 or with a dedicated Key Distribution Center. The other game computer 14 uses the public key to authenticate the Authenticatable Outcome Message AOM using the same protocol described above. Thus, the person seeking to authenticate the outcome simply calls the 800# and obtains the public key for the player alleging to have the outcome represented in his or her Authenticatable Outcome Message AOM. If the secure CPU 302 within the secure perimeter 300 is considered to be another "computer," the practice of certifying an outcome on the same game computer 14 that utilizes a secure CPU 302 to perform all encryption/decryption and/or authentication procedures, falls within the definition of mutual-authentication.

Referring now to FIGS. 14-28, another aspect of the invention facilitates "pay-per-use" in the home video game environment. This implementation confers several advantages. It enables any game computer 14 to be turned into a video game arcade machine. Players simply pay per game, or for play over a specified period of time. It also

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allows for specialized game computers 14 (game consoles) to be, in effect, "leased" to players. This has significant commercial implications, as many of the newer game consoles 14 have relatively high acquisition costs. This "time-dependent disablement" aspect of the invention permits players to acquire such game consoles 14 for a relatively low down payment. Charges for game play may then be incurred on a daily, weekly, monthly, or some other periodic basis. There are several embodiments of this aspect of the invention as described hereinafter.

In a preferred embodiment, the pay-per-use metering system utilizes cryptographic protocols to facilitate secure operation. The preferred method whereby unauthorized use of metered software is prevented employs encryption of part or all of the subject program. This program may be considered to be either the operating system that runs the game computer 14 (if use of the game computer 14 itself is to be metered), or any particular game program 26. A game program 26 will typically reside in an insecure data source such as a game cartridge, CD-ROM, hard disk, or the like. An operating system program 702 may reside in an insecure data source such as ROM and/or a hard disk associated with the game computer 14. The insecure data source is designated generally by the reference numeral 704.

The metering functions are implemented by a secure device having a secure perimeter. As described above, the secure perimeter is a defined physical area of hardware which is tamper-resistant and/or tamper evident. In this particular application, the secure device is referred to as a "meter" 502. As shown in FIG. 14, the meter 502 is a computer itself, and communicates with the game computer 14 to meter use/operation of a particular game program 26 or the game computer 14 itself via the operating system program. These types of programs are collectively referred to hereinafter as "metered programs 503", except where necessary to differentiate between them. In the inventive

system, the secure portions of metered programs 503 from the insecure data source 704 are decrypted and executed on the meter 502.

The meter 502 can be configured as part of the internal structure of the game computer 14, disposed on, for example, the motherboard, an expansion slot or the like. The meter 502 can be incorporated into a PCMCIA card such as the iPower card described above. Alternatively, the meter 502 can simply be a separate box that communicates with the game computer 14 via a data cable. Interfacing between the meter 502 and the CPU 27 of the game computer 14 can be implemented in a conventional fashion. The meter 502 includes a secure CPU 504, some non-volatile memory 506 such as a hard disk or flash ROM, ROM 508, RAM 510, a real-time clock chip 512, and a power source with battery backup 514. The meter 502 further includes an I/O port 515 for attaching a data communications cable to the game computer 14. In accordance with known techniques, the pins of the I/O port 515 can be electrically isolated to prevent pin-level probes. Similarily, the hardware components can be made with mechanical and chemical protection to prevent chip-probing equipment from accessing information from the secure CPU 504 directly. The non-volatile memory 506 may be used to store program instructions for implementing the overall metering functions. The ROM 508 contains encryption algorithms. The RAM 510 contains the cryptographic data and keys required to decrypt the secure portion of the metered program 503 to enable it to run, and for generating authenticatable messages that are communicated to the central computer 12 in connection with the metering functions, or game outcome authentication. If the meter 502 is tampered with, the encryption keys and data in the RAM 510 are erased from memory.

If the meter 502 is a separate unit, it can have input controls 516 to permit the player to manually enter codes directly into the meter 502 for authorizing metered

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software/game computer usage as described in more detail below. The meter 502 can also be configured with a display 518 to enable assorted information to be displayed to the player, including specifics relating to metered usage such as costs, authorized time periods, messages representing

5    information to be provided to the central computer 12 for authorization to run metered programs and/or Authenticatable Outcome Messages AOMs for games played on the game computer 14 that are manually entered into a telephone for communication over a telephone network as described above.

10   To facilitate communications directly between the meter 502 and the central computer 12, the meter 502 may have its own modem 520. Alternatively, the meter 502 can communicate with the central computer 12 via the modem 20 associated with the game computer 14.

15           Generally, each metered program 503 is encrypted using a key unique to that program. Alternatively, a single key may be used to encrypt a large number of metered programs 503, but such practice increases the security risk, since knowledge of that key (if it were somehow compromised)

20   could render an entire set of metered programs 503 which use it insecure. Therefore, it is anticipated that in most cases each metered program 503 is to be encrypted with a unique key. In order to run such a metered program 503, therefore, it is necessary for the metering system to acquire the key

25   for that metered program 503. This step can be made part of an "Adding a New Program" protocol to be described in more detail below. At the same time, it may be convenient to acquire an updated cost table including information for the new metered program 503, in systems implementing variable

30   costs. In this manner, the most current cost information will be available for running the new metered program 503. In some instances where it is desirable to have the convenience of running a metered program 503 for the first time without having to interact with the central computer

35   12, the meter 502 must already have a key for that metered

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program 503. This may be facilitated by having all such
metered programs 503 encrypted with the same key, where that
key is incorporated into all meters 502 in the system during
initialization thereof. Security of the system can be
further enhanced by using several different keys for the

5    metered programs 503, with key selection based upon the
Software_ID, a unique ID associated with each metered
program 503 (discussed in more detail below). In this
manner, the keys are equally shared across all such
immediately-runable metered programs 503, reducing the value

10   of each individual key of this type.

          In the inventive system, the metered programs 503
are classified as either of two types:
"immediately-runable" (and hence encrypted using one of the
shared keys); or requiring interaction with the central

15   computer 12 prior to the first time of execution on the game
computer 14. In the case of the latter, the metered program
503 is encrypted with a unique key. The immediately-runable
metered programs 503 utilize inherent cost information that
comes from the insecure data source 704 (at least during the

20   first billing period).

          Encryption:

          All messages between the central computer 12 and
the meter 502 are encrypted and authenticated. As discussed
in the foregoing with regard to outcome authentication,

25   among the various protocols, public-key or symmetric
encryption can be used, although symmetric encryption
appears to provide sufficient security for most of the
protocols. Such encryption may utilize key sizes in the
range of 64 to 128 bits. Examples include DES, 3DES, or

30   IDEA. Public key encryption most commonly employs RSA.
After initialization, the central computer 12 and the meter
502 share SK_Meter, which can be used with a conventional
encryption system to provide for both encryption and
authentication. Because the meter 502 has limited access to

35   sources of entropy, and because the total volume of data to

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be communicated between meter 502 and central computer 12 is small, a few hundred bytes per month in typical usage, using SK_Meter as the key for all communications between the two systems should provide adequate security for this application. In this configuration, messages from the meter 502 are preceded by sending ID_Meter (a unique identifier specific to the meter 502 and burned into its ROM 508 during manufacture) in the clear, allowing the central computer 12 to look up the encryption key used, followed by the message itself encrypted with SK_Meter. Responses from the central computer 12 are encrypted with SK_Meter for transmission to the meter 502.

All communications between the central computer 12 and meter 502 are initiated by the meter 502, with the central computer 12 acting as a server. As described in more detail below, it is expected that the player will actually initiate such communications rather than having the meter 502 spontaneously issue requests. Messages sent by the meter 502 may include a sequence number which will increment each time a message is sent in that direction. Reply messages from the central computer 12 include that same sequence number. This allows both sides to detect message replay attacks, in which messages are captured and then replayed at a later time in order to disrupt the protocols. The packet formats shown below do not include encryption headers or the account and sequence numbers, which are included as described above except where indicated. Each packet begins with a unique identifier value describing the kind of packet it is, and is followed by data as described below.

The following is a summary of data associated with the meter 502, and with each metered program 503:

Meter Keys:

The meter 502 utilizes a plurality of cryptographic keys to implement its functions, including secure communication with the central computer 12. As

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described above, these keys are of generally of three types: "secret" keys such as those used with conventional cryptographic protocols (e.g., DES); and "private" keys and "public" keys that are used with public key cryptographic protocols(e.g., RSA):

SK_Meter: The meter 502's secret key, also known to the central computer 12. This key enables secret and authenticated communication between the central computer 12 and the meter 502, possibly via an insecure communications link (a typical data network). This key is also employed by the meter 502 to create secure files out of insecure non-volatile storage associated with the game computer 14, by signing the data in the files using this key. SK_Meter is generated by the meter 502 during initialization, and is then transmitted to the central computer 12 and secured with PK_CC.

PRK_Meter: The meter's private key.

PBK_Meter: The meter's public key.

PK_CC: The central computer 12's public key, known to the meter 502. This key is used for initial communications between the meter 502 and the central computer 12 prior to creation of SK_Meter, and is burned into ROM 508 during manufacture.

SK_Imm_Run: The secret key for immediately-runable metered programs 503. As described above, it may be desirable to support metered programs 503 that can be run immediately upon acquisition, without running the Adding a New Program protocol described below. In this connection, the meter 502 must already have the key for this type of metered program 503. Accordingly, all such metered programs 503 share a special Software_ID, and the meter 502 recognizes that ID and uses the SK_Imm_Run key to decrypt a secure portion of the metered program 503, as described below in the Using Metered Software protocol. As described above, a variation on this defines several Software_ID's of the immediately-runable class, each of which is associated

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with a different SK_Imm_Run key.

ID_Meter: An identification number unique to each meter 502, burned into its ROM 508 during manufacture.

Account_Number: A number associated with the player who is responsible for payment for metered programs 503 that are operably enabled by the meter 502. This is the identifier which the central computer billing service uses to identify the player.

PIN: The player's personal identification number.

Limit_Time: A time/date value specifying the time limit beyond which metered programs 503 cannot run until receipt by the meter 502 of an authorization message from the central computer 12 for an additional amount of time.

Software_Key_Table: A list of Software_ID, Software_Key pairs. Each contains the key required to decrypt the encrypted portion of the metered program 503 with the specified Software_ID.

Meter Variables (If the meter 502 calculates costs):

Limit_Cost: If the meter 502 calculates costs associated with use of the metered program 503, a cost value specifying the maximum billable amount which can be accumulated during the current billing period, above which metered programs 503 cannot be run until receipt by the meter 502 of an authorization message from the central computer 12 for additional credit.

Total_Cost: The total amount of charges during the current billing period.

Cost_Table: Format to be described below.

Meter Variables (If the central computer 12 calculates costs):

Use Count Table: a list of Software_ID, Use_Count pairs, recording the number of times each metered program 503 or feature of a program was used during the current billing period. Software_ID's will be described below.

Use Time Table: A list of Software_ID, Use_Time pairs, recording the total amount of time each software

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package was used during the current billing period.

Software from Insecure Data Source:

In the illustrative embodiment shown in FIG. 15, each metered program 503 from the insecure data source 704 is divided into three parts: a Software Control Block 706,
5  an Insecure Software Component 708 and a Secure Software Component 710. The Software Control Block 706 contains software specific information that identifies metered program 503 to the meter 502 to enable the latter to calculate billing costs. The executable software itself
10  occupies the two remaining parts, where the Secure Software Component 710 is configured to run securely on the meter 502, and the Insecure Software Component 708 is designed to run in the insecure environment of the game computer 14.

Software Control Block 706: Information about the
15  software which will be used by the metering system to run it. The Software Control Block 706 is signed with the private key of the central computer 12, and the meter 502 verifies that signature when the software is loaded. Software Control Block 706 fields include:

20                Software_ID:  a  unique  number
              identifying  the  particular  metered
              program 503. Each metered program 503
              and  revision  thereof  has  a  unique
              Software_ID.  There  are  two  general
25                kinds  of  Software_ID's, "program" and
              "component", distinguishable  by  their
              high order bits.  Program Software_ID's
              are used for metered programs 503 which
              do  not  charge  per  feature,  while
30                Component  Software_ID's  refer  to
              specific features of a metered program
              503 and allow charging to be incurred
              on  the  basis  of  those  features.
              Component Software_ID's consist of two
35                fields,  a "major" and  a "minor"  ID

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component. The Software_ID_Major field identifies the metered program 503, and the Software_ID_Minor field identifies the particular chargeable feature of the metered program 503.

5     Software_Cost: This is the "base" cost information for this software component, in the form of a cost table (cost table formats are described below). Systems which do not support

10     variable costs will use the cost information from this table directly; those which support variable costs may have received override information from the central computer 12.

15     Insecure Software Component 708: The bulk of the metered program 503 that is executed by the game computer 14. It may be stored in encrypted form in the insecure data source 704, in which case the meter 502 decrypts it prior to being loaded into the RAM of the game computer 14. If this

20 decryption step will add unacceptable delay to program loading, the Insecure Software Component 708 can be stored unencrypted at only a slight loss of security. Since the memory of the game computer 14 is (by definition) insecure, a determined attacker can gain access to the plaintext of

25 the Insecure Software Component 708 in any case. Accordingly, the additional security added by storing it in secure form is limited in value.

    Secure Software Component 710: The Secure Software Component 710 is embodied in encrypted format in the

30 insecure data source 704, and must be loaded into and decrypted by the meter 502 when the rest of the metered program 503 is loaded into the RAM of the game computer 14. The Software Control Block 706 may also be loaded into the meter 502 at this time to facilitate charging for use of the

35 metered program 503. As will be described below, execution

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of the Secure Software Component 710 in the meter 502 implements selected but crucial functional operations on which the larger body of software in the Insecure Software Component 708 depends. The Secure Software Component 710 includes a secure routine that receives at least one input parameter from the game computer 14 when the Insecure Software Component 708 is executed. The secure routine is executed on the meter 502 to produce at least one output parameter. The at least one output parameter is communicated back to the game computer 14 from the meter 502, and the Insecure Software Component 708 is then executed on the game computer 14 with the at least one output parameter. For example, a game program 26 that directs the game computer 14 to generate a golf game, may include a secure routine comprised of instructions that direct the meter 502 to compute a resultant ball position in response to a plurality of input parameters. The input parameters to this routine may be the current ball location; type of club selected; force, direction and timing of the swing; and wind speed and direction. These input parameters are communicated from the CPU 27 of the game computer 14 to the meter 502 at the appropriate time. The secure routine is then executed on the Meter 502 to produce at least one output parameter, in this instance, a new ball position. This output parameter is then communicated back to the game computer CPU 27 and utilized by the Insecure Software Component 708 to display the new ball position in accordance with conventional practice. Thus, the game program 26 cannot be completely executed on the game computer 14 without receiving the required output parameters from the meter 502. Of particular significance, the instructions that make up the secure routine never reside in unencrypted form outside the meter 502. Only the result produced by executing such instructions in the meter 502 is returned to the RAM of the game computer 14. This prevents a player from reading the unencrypted instructions in the RAM of the game computer 14, and then using those

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unencrypted instructions to replace the encrypted instructions stored in the insecure data source 704 to circumvent the meter 502. Thus, encrypting the Secure Software Component 710 and executing the secure routine therein on the meter 502 is the mechanism by which the overall security of the system is maintained. It prevents attackers from deducing and replacing the functionality of this software component in order to circumvent the system and execute metered software without paying for it. The meter 502 enables operation of such metered programs 503 until the Limit_Time or Limit_Cost is reached.

The foregoing arrangement may be used to meter use of the game computer 14. When the game computer 14 is turned on, the Secure Software Component 710 of the operating system program 26 is loaded into the meter and decrypted. In order to run the game computer 14, the Insecure Software Component 708 of the operating system program in the game computer 14 must receive at least one output parameter from execution of the secure routine in the Secure Software Component 710 in the meter 502 that is essential to proper functioning of the complete operating system program. Without receiving the required at least one output parameter, the game computer 14 cannot execute any programs. The at least one output parameter from the meter 502 may be required at specific times during the operation of the game computer 14. When the Limit_Time or Limit_Cost for metered usage is reached, the meter 502 disables operation of the game computer 14 by no longer providing the at least one output parameter crucial to the Insecure Software Component 708 of the operating system on the game computer 14 the next time it is required. Since the system may be designed such that the at least one output parameter from the meter 502 is required by the game computer operating system program at very short time intervals, the time delay between the meter 502 ascertaining that metered usage is to be suspended and the actual suspension of game computer operation by not

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providing the requisite at least one output parameter from the meter 502 the next time it is required, is effectively so small such that, for all practical purposes, operation of the game computer 14 is suspended immediately after the cost or time limit is exceeded.

In an alternative embodiment, the Meter 502 has the Secure Software Component 710 for any game program 26 or an operating system program permanently stored in its own secure memory instead of the insecure data source 704. Thus, the Secure Software Component 710 need not be encrypted since players cannot gain access to such software instructions. Security is provided by the tamper-resistant meter configuration. As described above, attempts to tamper with the secure memory to read the Secure Software Component 710 result in a memory loss of the Secure Software Component 710. Depending upon the configuration of the meter 502, the secure memory can be adapted to store a plurality of Secure Software Components 710, each for a different metered game program 26 or operating system program, or a generic Secure Software Component 710 which can be used with a plurality of game programs 26 or operating system programs.

Cost Tables:

The cost table data structure is used by the Software_Cost element of the Software Control Block 706 to enable charges to be made for the base costs of software, and is also stored by the meter 502 in non-volatile memory for systems which support variable costs. It is a list of Cost_Table_Elements, of the following format:

Software_ID
Charging_Method
Charge_Per_Use
Charge_Per_Time
Override
Base
Multiplicative
Replacement

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Charge Value

Per-Minute or Per-Use.

Each cost element relates to a particular Software_ID. If it is a "program" Software_ID, the cost table element refers to billing for the program itself, either on a per-minute or per-use basis. If it is a "component" Software_ID, the cost table corresponds to a cost for using that particular feature or component of the software (i.e., the charge for execution of a game program may relate to certain "features" of the game that are implemented as a result of game play, such as for example, reaching a certain level or expending a given number of lives and the like). Typically this may be on a per-use basis, but in some cases special charges might apply on a per minute basis when certain components or features are used, such as when the player engages a particular enemy or uses specific weapons at his disposal.

The Charging_Method is one of the two identifiers Charge_Per_Use or Charge_Per_Time to indicate which form of charging is to be applied when the Software_ID is used.

The Override field indicates how this charge table element is to be combined with other cost tables, if they exist. If it has the value "Base" that means that this is a base cost table element, usually associated with the Software Control Block 706 in the insecure data source 704. If there are no other charge table elements for this Software_ID then this element's cost data will be used. The other two possible values are used for cost tables which are downloaded by the central computer 12 to change the cost values stored in the insecure data source 704. "Replacement" means that this cost table element overrides and replaces any base cost table element from the insecure data source 704. "Multiplicative" means that the cost values are fixed-point numbers which multiply the cost values from the Base cost table and adjust them.

Lastly, the Charge_Value is represented in units that are implementation-dependent and country-dependent, but

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which refer to each use or each minute of use of the feature, for the Base and Replacement Override values. For the Multiplicative case the Charge_Value is a fractional value as described above which multiplies the Base cost value.

Receipt File 712:

If insecure non-volatile memory (e.g., a disk drive) is associated with the game computer 14, then some of this memory can be used to record detailed information about the billing activity during a billing period. This information is referred to as a Receipt File 712, and contains a series of receipt entries, each of which reflects some billing action. Although the Receipt File 712 is stored in insecure memory, the file entries are signed by the meter 502 to ensure their integrity and accuracy. The Receipt File 712 provides detailed information to the player about his billings, and also can be used to aid in dispute resolution where the player claims that his bill from the central computer 12 does not match his usage.

Meter 502/Game Computer Interface:

As described above, the Insecure and Secure Software Components jointly implement the functionality of the metered program 503. The bulk of the metered program 503 is the Insecure Component, but it makes use of the Secure Component that is decrypted and executed on the meter 502 to implement the metered program 503 on the game computer 14. The meter 502 will generally be a much less powerful processor than the game computer 14. It also may not have access to the game computer's memory and internal data structures. Accordingly, data to be exchanged therebetween can utilize a message-passing or subroutine call interface. In addition, the Secure Software Component 710 can be designed so that it performs crucial and non-trivial parts of the calculations implemented by the Insecure Software Component 708 of the metered program 503. In general, any event which may generate a billing charge

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must involve the cooperation of the Secure Software Component 710. The calculations done by that part must be non-trivial to prevent attackers from reverse engineering the Insecure Software Component 708 and replacing the calls to the Secure Software Component 710 with local code that

5  provides an equivalent function. This task is aided by the encryption of the Secure Software Component 710, which requires attackers to deduce the purpose of the code by observing its behavior from the insecure side. However, to effectively thwart such an attack, the Secure Software

10  Component 710 must be sufficiently complex. In an embodiment where the meter 502 is disposed between a CD-ROM and the game computer 14, access control is made easy as the metered program data must be decrypted on the fly by the meter 502. The interface between the two parts, in an exemplary

15  embodiment, is a subroutine call interface, in effect a remote procedure call since the code is being executed on another processor. In the preferred embodiment, the Secure and Insecure Software Components operate concurrently, with the Secure Software Component 710 performing a calculation

20  whose result will be needed by the Insecure Software Component 708 at some point in the future. One attack which must be prevented, is the player turning off power to the system at an inopportune moment. For example, if software is to be billed by the amount of time spent, and if such

25  billing was implemented by inserting calls to the Secure Software Component 710 at the beginning and ending of the program (with the intention that the Secure Software Component 710 calculate the elapsed time by taking the difference between the time of the two calls), then billing

30  might be avoided by always turning off the power before the program normally terminated. One way to avoid this is to record the progress of time incrementally as certain programmed events occhur. Incremental charging provides fewer opportunities to evade billing by these kinds of

35  methods. Charging by use or by feature is also less

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vulnerable to this attack. Another alternative, is to note in the audit logs the time when the power is cut prior to game termination. A pattern of games that have no end-of-game record signifies a player trying to evade the system.

Protocols:

The meter 502 communicates with the central computer 12 at regular intervals to report usage information, receive authorization for additional usage, receive updated cost information, and resolve disputes. Communications between the meter 502 and the central computer 12 can be automatic (without player intervention), but most players may be more comfortable with a system in which they control when such communications occur.

Several different communication protocols are utilized to facilitate operation of the metering system. Some employ communications between the game computer-meter system and the central computer 12, while others relate to purely local transmissions between the game computer 14, meter 502, and insecure data source 704. These protocols include:

Initialization of the Meter 502 (FIG. 16): This protocol is implemented when the player initializes the newly purchased meter 502 or metering system. The meter 502 performs its own internal initialization, including communicating with the central computer 12 to generate shared cryptographic keys. At this stage, the meter 502 has access to PK_CC, the central computer's public key, and ID_Meter, its own unique ID. The meter 502 is capable of generating random numbers. Unlike other protocols, these packets are not implicitly encrypted with the keys shared between central computer 12 and the meter 502. Instead, the encryption step is explicitly identified at each step of the following illustrative protocol:

At step 522, the meter 502 calculates SK_Meter, the random key for communicating data between itself and the

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central computer.

At step 524, the meter 502 creates an Initialization Message block of the following format:

Initialization Message
ID_Meter
Current date and time
SK_Meter.

At step 526, the meter 502 encrypts the Initialization Message block with SK_Meter, then encrypts SK_Meter using PK_CC and communicates this encrypted message to the central computer 12.

At step 528, the central computer 12 recovers SK_Meter and then the Initialization Message block. It verifies that the date and time are approximately current, and records the new ID_Meter, verifying that it has not been used before. It recalls SK_Meter, and associates that value with ID_Meter.

At step 530, the central computer 12 creates an Account_Number that will be associated with the player who owns the meter 502 and pays for the software.

At step 532, the central computer 12 creates an Initialization Response Message block of the following form:

Initialization Response Message
Account_Number;

At step 534, the central computer 12 encrypts the Initialization Response Message block under SK_Meter and sends it back to the meter 502.

At step 536, the meter 502 decrypts the Initialization Response Message block and stores the Account_Number. It then displays the Account_Number to the player for his records and use in communicating with the central computer billing service when he opens his account;

At step 538, if the meter 502 has an associated clock chip, the meter 502 runs the Synchronizing Clock protocol.

After the player has opened his account with the

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central computer billing service, go to the Authorization from Central Computer protocol described hereinbelow.

Adding a New Program (FIG. 17): This protocol is used when the player acquires a new software program that requires information from the central computer 12 in order to run.  As will be described below, some programs may be runable without any new information from the central computer 12, but others may require that new cryptographic keys for those specific programs be acquired. All messages transmitted in this protocol are  protected by encryption and sequence numbers as described above. An exemplary protocol operates as follows:

At step 540, the meter 502 reads new programs' Software Control Block(s) from the insecure data source 704, and extracts the Software_ID for each program.

At step 542, the meter 502 creates a New Program Message in the following format:

> New Program Message
> Number of programs requested
> Software_ID
> Software_ID
> . . .

At step 544, the meter 502 securely transmits the New Program Message to the central computer 12.

At step 546, the central computer 12 looks up the Software_ID's for the keys that are requested to determine the keys needed to decrypt those programs.

At step 548, the central computer 12 creates a New Program Message Response block in the following format:

> New Program Message Response
> Number of Programs Requested
> Software_ID, Key
> Software_ID, Key
> . . .

At step 550, the meter 502 records the key information for each software program in its

Software_Key_Table structure.

At step 552, the meter 502 runs the Updating Cost Information protocol if the variable cost option is being used.

Authorization from the Central Computer 12 (FIG. 18): The central computer 12 transmits authorization data to the meter 502 to enable operation for a specified time period Limit_Time or until a specified amount of money Limit_Cost is reached, as discussed above. Normally this is implemented at the end of the billing period, immediately after running the Reporting Usage protocol. Alternatively, this may be done to obtain a specified amount of credit, after the exhaustion of which the meter 502 requires additional authorization. It is also done after the account is set up for the first time. Here again, all messages communicated in this protocol are protected by encryption and sequence numbers as described above. An illustrative protocol operates as follows:

At step 554, the meter 502 generates an Authorization Request Message, of the following format, which is sent securely to the central computer 12:

>Authorization Request
>Current Date/Time
>Limit_Time
>Limit_Cost (if meter 502 calculates costs)
>Total_Cost (if meter 502 calculates costs).

At step 556, the central computer 12 records the time and cost information for statistical purposes. It then determines whether the current account (based on the Account_Number) is paid up pursuant to whatever billing conventions are being utilized to authorize continued use of the game computer 14 and/or software via the meter 502. Based on this, it then calculates new values for Limit_Time and Limit_Cost. If no additional authorization is approved, then the values in Limit_Time and Limit_Cost are left unchanged.

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At step 558, the central computer 12 creates and sends an Authorization Response Message block to the meter 502 in the following format:

> Authorization Response
> Current Date/Time from previous message
> New Limit_Time value
> New Limit_Cost value (if meter 502 calculates

costs).

At step 560, the meter 502 verifies that this Authorization Response Message is not a replay (i.e., the message is "fresh") by checking the time stamp. It then copies the new Limit_Time and Limit_Cost values into its non-volatile memory.

<u>Updating Cost Information (Optional - Variable Costs) (FIG. 19)</u>: This protocol is used when the meter 502 requires updated cost information from the central computer 12 for metered programs 503 that the player is currently using. It is only employed if the variable cost option is selected, and is run after the Adding a New Program protocol, as well as during the regular set of protocols run at the end of each billing cycle (with the Authorization from Central Computer and Reporting Usage protocols). All messages communicated in this protocol are protected by encryption and sequence numbers as described above. An illustrative protocol is described in the following:

At step 562, the meter 502 creates a Cost Update Request Message and sends it to central computer 12 in the following format:

> Cost Update Request
> Software_ID
> Software_ID
> . . .

At step 564, the central computer 12 creates a Cost Update Response Message containing cost information for the requested Software_ID's. For those Software_ID's which include per-feature billing, cost information for such

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individual features are also included. This information is provided in the form of a Cost Table as described above:

Cost Update Response

Cost_Table.

At step 566, the meter 502 merges the new Cost_Table information into its internal Cost_Table, replacing internal data for matching Software_ID's.

Synchronizing Clock (FIG. 20): This protocol is run at regular intervals to synchronize the clock 512 in the meter 502 with the one in the central computer 12. Typically, this may occur at the end of every billing period. In this manner, the two (2) components are in agreement on the time limits at which authorizations will expire. (This protocol can be designed to handle propagation delays if it is expected to work on asynchronous data network systems like the Internet.) An exemplary protocol follows:

At step 568, the meter 502 creates a Time Update Request Message, of the following format, which is then securely communicated to the central computer 12;

Time Update Request

Current Date/Time from Meter.

At step 570, the central computer 12 reads the meter 502's date/time, which is provided for statistical analysis purposes. It then replies with a Time Update Message to the meter 502, which includes the current time in the packet format:

Time Update

Date/Time from Meter (taken from previous message)

Current Date/Time from Central Computer;

At step 572, the meter 502 receives the Time Update packet, checks the sequence numbers and encryption, and notes the time, but does not yet update it. It also records the time this data packet was received, based on its own clock. It also verifies the date/time from what it

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communicated to the central computer, and then returns a
Time Update Acknowledgement Message to the central computer,
of the format:

> Time Update Acknowledgment
>
> Current Date/Time from Central Computer.

5        At step 574, the central computer 12 compares the
time between when it sent the Time Update packet and when it
received the Time Update Acknowledgment packet. If the time
is within a specified period of accuracy, it then sends a
Time Update OK Message, of the form:

10       Time Update OK
>
> Repeat of Original Date/Time from Central
> Computer.

At step 576, upon receipt of the Time Update OK
Message, the meter 502 updates its time, based on the time
15   in the Time Update Message and its own calculation of the
elapsed time between the receipt of the two (2) Messages
from the central computer 12. The value it sets its own
time base to is the Date/Time value from the central
computer 12 plus the difference in time between the arrival
20   of the Time Update and Time Update OK Messages.

Starting Metered Software (FIG. 21): This protocol
involves the meter 502, game computer 14, and insecure data
source 704, and their interaction when a metered program is
loaded for execution.

25       As described above, a metered program 503 that
resides in the insecure data source 704 contains a Software
Control Block 706 and two executable components, an Insecure
Software Component 708 which executes on the game computer
14 and a Secure Software Component 710 which is decrypted
30   and executed on the meter 502. At least the Secure Software
Component 710 is encrypted, although the Insecure Software
Component 708 and the Software Control Block 706 may be
encrypted as well. The protocol operates as follows in a
sample embodiment where the Software Control Block 706 is
35   signed by the meter 502 and the Secure Software Component

710 is encrypted:

At step 578, the meter 502 reads the Software Control Block from the insecure data source 704, verifies the signature of the Software Control Block, and extracts the Software_ID for that metered program 503.

At step 580, the meter 502 determines whether the required key is available to decrypt the Secure Software Component 710 of the metered program 503. This key is found either by looking up the Software_ID from the Software Control Block 706 in the Software_Key_Table, or may consist of SK_Imm_Run for immediately-runable programs (recognized by the associated Software_ID). If the key is unavailable, the meter 502 displays a message informing the player that the new program must be added to the current software list, and that communication with the central computer 12 is required to obtain the necessary keys (and this protocol terminates).

At step 582, if the required key is found, the meter 502 then determines whether the current resource limits are sufficient to run the metered program 503. If Limit_Time or Limit_Cost exceeds a predetermined value, then a message is displayed that advises the player that he must communicate with central computer 12 to authorize additional usage of metered software (and this protocol terminates).

At step 584, if either the Limit_Cost or Limit_Time are in a certain range near the maximum predetermined value, the meter 502 can display a warning before proceeding, informing the player that he may lose authorization during the course of running the metered program 503, and requiring confirmation by the player to continue, otherwise this protocol terminates.

At step 586, the meter 502 reads the Software_Cost_Table from the Software Control Block 706, and applies any overrides or adjustments from its internal Cost_Table, saving the resulting data in its internal RAM 510.

At step 588, the meter 502 and game computer 14 read the Secure Software Component 710 and the Insecure Secure Software Component 708, respectively, from the insecure data source 704. The meter 502 decrypts the Secure Software Component 710.

5          At step 590, the meter 502 and game computer 14 then transfer control to the newly read software components, with the meter 502 running the Secure Software Component 710 and game computer 14 running the Insecure Software Component 708.

10          Running Metered Software (FIG. 22): The following discussion relates to the interaction between the meter 502 and game computer 14 in connection with billing during execution of the program.

As described above, the meter 502 and the game
15    computer 14 respectively execute the Secure Software Component 710 and the Insecure Software Component 708 of the metered program 503. In order to ensure accurate billing, the dependencies between the two components must be designed so that all events which are billable require the active
20    cooperation of the Secure Software Component 710. When one of these events occurs, this protocol is run. Furthermore, if non-volatile storage is available on the game computer 14, the meter 502 can use this storage to maintain the Receipt File 712, which provides detailed itemization of
25    billing activity. The Receipt File 712 constitutes a record of the player's activity and, since it is signed by the meter 502, it can be used in resolving any disputes. The following is an illustrative protocol; note that Steps 592 and 594 thereof are executed only in case a Receipt File 712
30    is available:

At step 592, the meter 502 creates a Receipt Entry consisting of the following information: the software being used, the amount charged (if available), the current time, and information to identify the charged event. This is
35    signed with the meter 502's secret key (i.e., SK_Meter if

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conventional cryptography is used, or via public key techniques). The meter 502 then sends the Receipt Entry to insecure storage, where it is appended to the Receipt File 712.

At step 594, the meter 502 updates the Total_Cost based on which event is being billed and the current cost information calculated during the Starting Metered Software protocol.

At step 596, if at any time during operation of the metered program 503, either the Limit_Time or Limit_Cost are exceeded, the meter 502 no longer executes the Secure Software Component 710 to produce the at least one output parameter required by the Insecure Software Component, thereby interrupting execution of the program on the game computer 14, and displays a message advising the player that he must contact the central computer 12 to obtain further authorization;

If appending a signature to each entry in the Receipt File 712 is deemed too labor intensive, Step 592 above can be replaced by the following sequence:

At step 592a, the meter 502 reads the current Receipt File 712 from insecure storage. The Receipt File 712 consists of receipt records as described above (however the individual records are unsigned in this case), followed by a signature relating to the file as a whole;

At step 592b, the meter 502 verifies the signature on the Receipt File 712. As described above, this may be implemented by using conventional cryptography using SK_Meter or public key techniques;

At step 592c, if the signature is valid, the meter 502 adds the new Receipt Entry to the Receipt File 712 and signs the entire file. The Receipt File 712 is then returned to insecure storage; If the signature is invalid, At step 592d, the meter 502 adds a line signifying that the above information is corrupted. It then appends the current Receipt Entry and signs the entire file. A message is

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displayed to the player indicating that the Receipt File 712 is corrupted.

Reporting Usage (FIG. 23): At the end of the billing period the meter 502 reports accumulated usage information to the central computer 12. Several variations are possible depending on which payment mechanism is in use, and the level of detail of the information to be communicated to the central computer 12. If the meter 502 is calculating the costs (in lieu of the central computer 12), the following protocol is used. As an option, a copy of the Receipt File 712 (if one is available) can be uploaded to the central computer 12.  If the information is uploaded, the central computer 12 can prepare a detailed printed billing statement similar in format to a typical telephone bill.  The following is a sample protocol:

At step 598, the meter 502 creates a Usage Report Message containing the following information:

  Usage Report

  Total_Cost

  Receipt File (optional).

At step 600, the central computer 12 receives the Usage Report Message and updates the billing information for this  account.

At step 602,  the central computer 12 returns a Usage Report Acknowledgment Message arranged in the following format:

  Usage Report Acknowledgment

  Total_Cost.

At step 604, the meter 502 displays the Total_Cost that is being billed.

At step 606,  the meter 502 signals the game computer 14 to rename the Receipt File 712 to a sequentially numbered  backup file, and creates a new blank Receipt File 712; Normally other protocols will be run at this time, including Authorization from the Central Computer, Synchronizing Clock, and Updating Cost Information.

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If costs are calculated by the central computer 12 instead of the meter 502, more detailed information must be uploaded:

At step 608, the meter 502 creates a Usage Report Message containing the following information:

Usage Report

Use_Count_Table

Use_Time_Table

Receipt File (optional).

At step 610, the central computer 12, knowing the costs of various programs and program features, calculates and updates the billing information for this account. It can also use this information to prepare an itemized statement showing how the various pieces of software contributed to the billed amount. It calculates Total_Cost, the total new amount being billed, and returns a Usage Report Acknowledgment Message of the form:

Usage Report Acknowledgment

Total_Cost.

At step 612, the meter 502 displays the Total_Cost that is being billed.

At step 614, the meter 502 signals the game computer 14 to rename the Receipt File 712 to a sequentially numbered backup file, and creates a new blank Receipt File 712; other protocols which would normally run at this time include Authorization from Central Computer and Synchronizing Clock.

Auditing (FIG. 24): This protocol is utilized when a disagreement regarding usage and billing arises between the player and the central authority, and is implemented with the Receipt File 712. A validly signed Receipt File 712 is an authenticatable record of all billing activity for any given billing period. If the player's bill from the central computer 12 does not match the information contained in the Receipt File 712, the following protocol may be followed:

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At step 616, the meter 502 creates a Receipt File Check Message in the following format:

Receipt File Check

Receipt File (from disputed billing period).

At step 618, the central computer 12 acquires the Receipt File 712 and compares it to the billing information it received from the meter 502 for that billing period (the billing period is deduced from the dates in the Receipt File 712). Any disagreement indicates a possible malfunction in the system; possibly a faulty meter 502, erroneous software, or operator error at the central computer 12. If the Receipt File 712 confirms the accuracy of the billing data, the player may have made a mistake in verifying his Receipt File 712.

At step 620, in either case, the central computer 12 creates a Receipt File Validation Message, including the original amount billed during the billing period in question, and the validated amount. These will be the same as the original amount if no discrepancies were found, or a corrected amount if an error was detected:

Receipt File Validation

Date/Time of Start of Billing Period

Date/Time of End of Billing Period

Original Amount Billed

Validated/Corrected Amount Billed

Null-Terminated Text String (for display to player).

At step 622, the meter 502 displays this information and the text string communicated from the central computer 12, thereby providing an explanation for any discrepancies that may have been found.

Authentication (FIGS. 25 and 26):

One application of the outcome authentication system described in detail in the foregoing, is "high score" verification in the context of a dedicated game system, or entertainment software on a general purpose

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computer. This is the principle protocol of the system, used when a program produces some outcome which the player wants to have authenticated. We assume that the division of software between the secure and insecure components is such that the meter 502 can in fact determine that the specified outcome actually occurred. For the electronic communication implementation shown in FIG. 25, an illustrative protocol operates as follows:

At step 624, the meter 502 creates an Authenticatable Outcome Message AOM of the following form encrypted with SK_Meter:

> Authenticatable Outcome
> Software_ID
> Null Terminated Text String (describing outcome for display to player).

At step 626, the central computer 12 receives and authenticates the block by decrypting the same using SK_Meter and, if authenticated, it then accepts the outcome on that basis. As described above, the central computer 12 may then re-authenticate the outcome under its own PK_CC or implement whatever other actions are appropriate.

At step 628, the central computer 12 returns an Authenticated Outcome Confirmation Message AOCM confirming that it has accepted the Authenticatable Outcome in the form:

> Authenticated Outcome Confirmation
> Software_ID
> Null Terminated Text String (confirmation or denial of outcome acceptance for display to player).

At step 630, the meter 502 displays the confirmation or denial of outcome acceptance from the central computer 12.

As described above, players can manually enter messages into a telephone keypad to facilitate communications with the central computer 12. In this

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situation, a different protocol may be used that is more amenable to the limited communications bandwidth associated with a manual transfer of data between the meter 502 and the central computer 12 as shown in FIG. 26:

At step 632, the meter 502 displays the game outcome on the screen, along with its ID_Meter (possibly just some fraction of the bits of ID_Meter is shown, enough to narrow down the source possibilities to no more than a handful of meters 502).

At step 634, the player dials the central computer 12 on the telephone and enters this information using his touch-tone keypad in response to suitable prompts from an IVRU as described in detail above.

At step 636, the central computer 12 generates and communicates a random challenge string to the player, who then enters the same using an appropriate input device (e.g., a keypad, joystick interface or the like).

At step 638, the meter 502 calculates a cryptographic hash of the game outcome and challenge string, encrypts it with SK_Meter, and displays the result-an Authenticatable Outcome Message AOM.

At step 640, the player enters the Authenticatable Outcome Message AOM into the telephone keypad from where it is communicated to the central computer 12.

At step 642, the central computer 12 authenticates the Authenticatable Outcome Message AOM by independently calculating the hash and encryption, and confirms the value received from the player.

At step 644, the central computer 12 returns an Authenticated Outcome Confirmation Message as described above. This may be accompanied by a verbal indication of outcome acceptance or denial by the IVRU.

At step 646, the player enters the Authenticated Outcome Confirmation Message into the meter 502, which displays the confirmation or denial of outcome acceptance as

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described above.

      Several variations of the foregoing are anticipated. To improve reliability, the values communicated from the player can be padded with some redundancy to allow some level of error detection if he enters a few digits incorrectly. A variation on the above protocol employs a key other than SK_Meter, which is the same for all the meters 502 and is programmed into their ROM 510 during manufacture. This obviates the need for the meter 502 to display ID_Meter at step 632 and the player to enter it at step 634. In addition, Step 638 can be alternatively accomplished by hashing in the secret key value rather than encrypting with it.

      In accordance with the foregoing, the metering protocols enable use of the game computer 14 or game program 26 to be metered in several ways, including: time-dependent disablement, credit requests to enable arcade-type play, and flat-rate pricing. Game play may be controlled by the meter 502 by not executing the Secure Software Component 710 of the game computer operating system, or not executing the Secure Software Component 710 of a game program 26 without authorization. In addition, game play can be metered using an alternative system where the game is not displayed in a usable or intended format without authorization. The latter can be accomplished by scrambling the game program instructions and/or data such that a descrambler 500 is required to descramble the game, where the descrambler 500 is enabled by an Authenticatable Unlock Message AUM. The descrambling embodiment is described separately below.

      In a time-dependent disablement embodiment, the game computer 14 is adapted to function for a specified period of time, after which it requires that an "unlocking code" be input to enable play after the expiration or end date. The player obtains an unlocking code (hereinafter referred to as an Authenticatable Unlock Message AUM) in order to continue play after the end date. The

Authenticatable Unlock Message AUM is obtained from the central computer 12 by implementing the Authorization from Central Computer protocol described above. The Authorization Request Message in that protocol is identified as an Authenticatable Unlock Request Message AURM, where the

5    Limit_Time value specifies the length of the next period of use to be purchased. The player may be provided with the option to purchase time by the month, week, day, etc. The player may provide a credit card for billing, or billing can be made at the end of the specified period in the Reporting

10   Usage protocol described above. In the Authorization from Central Computer protocol, the central computer 12 authenticates the Authenticatable Unlock Request Message AURM, checks the status of the player's account, and generates an Authenticatable Unlock Message AUM which

15   constitutes the above-described Authorization Response Message, where New Limit_Time represents the authorized period of time. After the meter authenticates the Authenticatable Unlock Message AUM, it changes the end date for usage represented in the New Limit_Time in its

20   non-volatile memory 506. During the authorized time period, charges may be incurred for different types of usage depending upon the Cost_Table_Elements for the game programs 26. Additional time may also be purchased by calling a 900#, or billing may be implemented by mail, or at a retail

25   outlet. For example, an AURM could be written down, printed out or stored on portable data memory media, and provided to an authorized agent at a terminal connected to the central computer 12. With specially configured metered software 503, players may have several options, such as a limited number

30   of minutes of play time, where every minute of play reduces the allotted play time by an equivalent value (e.g., one minute) or by some other value. When the allotted time expires, the meter 502 no longer operates to execute the Secure Software Component 710, thereby rendering the game

35   computer 14 or a particular game program 26 inoperative. In

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this connection, it is anticipated that play may be restricted to certain hours of the day, weeks in the month and the like. For example, the meter 502 may prevent game play between the hours of 11pm and 7am to prevent children from playing late into the night. Rates for enabling play can be varied depending upon the time or day of play as well.

In an arcade-type embodiment, the player purchases "credits" to enable game play. This enables players to call the central computer 12 and obtain codes for a specified number of game plays, as in an arcade environment. The methodolgy is similar to the foregoing embodiment, except that the Authorization Request Message to the central computer 12 is identified as an Authenticatable Credit Request Message ACRM. The Authenticatable Credit Request Message ACRM represents a specified number of game plays that are requested from the central computer 12. In the Authorization from Central Computer protocol, the central computer 12 generates an Authenticatable Credit Authorization Message ACAM which constitutes the above-described Authorization Response Message, where New Limit_Cost represents a number of play credits. After the meter authenticates the Authenticatable Credit Authorization Message ACAM, it copies the credit amount Limit_Cost into its non-volatile memory 506. Each play results in the credit amount being decremented in the meter 502. When the purchased credit amount is exhausted, if the player desires to continue to play, he must purchase more credits from the central computer 12 via the Authorization from Central Computer protocol.

Each game to be played may decrease the total credit value by a specified amount. Popular games may be made to require two or more "credits" per play. Some games may be designed to accept additional credits once a player has lost all of his or her allotted lives (e.g., such as with video arcade machines), thereby enabling the player to

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continue the game. Extra credits can be required to complete restricted stages of a game. A player might be charged one credit to explore any of the first ten levels of a game, while any of the additional five bonus levels could be explored at a cost of one credit each. For example, while exploring a ten-level dungeon, the player could be charged an additional credit for the use of a special weapon, access to a map of the dungeon, or hints on avoiding traps. Such extra characteristics may be implemented in the Software Control Block 706 for the particular game program 26, and may be configured so as to require additional credits in the meter 502 to continue play at these levels. The number of credits available may also be incremented after achieving a certain level of performance in the game. For example, scoring over a million points in DONKEY KONG might result in an extra credit being added to the available credit balance. Finding a secret room within a game might add five credits. Hitting a home run may earn ten credits. This information can then be stored in the volatile memory of the meter 502 so that this five credit bonus cannot be repeated in future games. The number of credits that a player receives per dollar may also be variable. A purchase of ten credits may cost $.50 each while a purchase of twenty credits may cost $.30 each. Credit discounts can be offered to select players who have obtained certain certified titles. A five-star MORTAL KOMBAT player may receive a 10% discount on all credits. Each credit might also buy a certain number of lives. Games may cost a different number of credits depending upon the difficulty setting. For example, the easiest setting may cost two credits while the most difficult setting may cost one credit per play. In lieu of purchasing one game, each credit may entitle the player to play for a certain period of time. One credit may buy five minutes of play while two credits may buy twelve minutes of play. As described above, the meter 502 can determine the price per game credit from the data or instructions

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associated with Software Control Block 706 of the game program 26. The price per game may be stored within the meter's 502 non-volatile memory and can be manipulated with codes from the central computer 12 to alter or change the pricing structure for particular games via the Updating Cost

5    Information protocol described above.

In lieu of charging a specified number of credits for each game play, it will be appreciated by persons skilled in the art that a flat rate for unlimited use over a fixed period of time may be implemented by requesting a

10   flat-rate pricing option from the central computer 12. The Authorization from Central Computer protocol is implemented as described in the foregoing, except that the Authorization Request constitutes an Authenticatable Flat Rate Request Message AFFRM, where Limit_Time represents a requested fixed

15   time period for unlimited usage. The central computer 12 authenticates the Authentictable Flat Rate Request Message AFRRM, checks the status of the player's account, and generates an Authenticatable Flat Rate Message AFRM, which constitutes the above-described Authorization Response

20   Mmessage where New Limit_Time represents authorization for unlimited usage for the requested time period. To operate the game computer 14 or to run a particular game program 26, the meter simply checks the New Limit_Time prior to and during each play as it executes the Secure Software

25   Component 710 of the operating system program or the game program 26.

As mentioned above, another method by which play can be prevented involves scrambling the game data in a particular game program 26 that causes the game computer 14

30   to generate the game with altered characteristics. A shown in FIG. 27, a descrambler 700 is either installed within the game computer 14 itself, or is connected to the video output cable from the game computer 14. In order to convert the scrambled game into the intended format, the descrambler 700

35   must be enabled by an Authenticatable Unlock Message AUM,

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Authenticatable Credit Authorization Message ACAM or Authenticatable Flat Rate Request Message AFRM as described above. By way of example, an illustrative mode of operation is hereinafter described.

5          Data and instructions associated with computer games typically contain a background element as well as game character data commonly referred to as sprites. A particular game may have a dozen different backgrounds representing a dozen different levels. An example of a background is a jungle scene depicting trees, plants, rivers, rocks, etc.

10       The game character that the player controls, as well as any enemy characters encountered, are normally represented by sprites, which are movable within the game background. This background may be analogized to a map, with respect to which the sprites travel. This "game data" is not to be confused

15       with the software code that is executed by the CPU 27. The game data in this context is used to generate the video output and may be processed by the CPU 27, even if scrambled or encrypted. The processed game data visual information is composed of pixels. Each pixel has a color and an associated

20       location for display. An illustrative pixel reference (34,15,26) may correspond to a blue color (indicated by the numeral 26) at a location residing at 34 pixels above the bottom of the screen (a horizontal datum) and 15 pixels to the right of the left side of the screen (a vertical datum).

25       This information is stored in the read only memory of the game cartridge. Normally, the game program 26 is executed by the CPU 27, converted by appropriate means into a video signal (RF format), and communicated to a display device such as a TV. In this embodiment, the video signal contains

30       altered characteristics, which must be applied to the descrambler 700 to make the output video signal represent the real game in the intended format. In the scrambling process, the pixel data may be algorithmically manipulated such that the pixel data does not represent the intended

35       format. For example, the intended pixel (34,15,26) may be

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transformed into (12,02,68). The transformed pixel data is
stored in the game cartridge and when processed, is
unintelligible to the player if displayed on the TV without
the intervening descrambling process. When the descrambler
700 is enabled with the proper Authenticatable Unlock
5    Message, it operates on the video signal to convert
(12,02,68) back to (34,15,26), the intended format. The
scrambling and descrambling process may utilize
algorithm/inverse algorithm pairs and/or the cryptographic
authentication protocols described herein. For example, the
10   value (12,02,68) is obtained by encrypting (34,15,26) with
a secret key, and subsequently authenticated with a public
key in the descrambler 700. The descrambler 700 will not
operate to descramble the video signal after the end date,
or prior to receiving the proper Authenticatable Unlock
15   Message AUM, Authenticatable Credit Authorization Message
ACAM, or authentictable flat rate message AFRM.

As a result of game play, reward points may be
accumulated in a manner similar to a frequent flyer reward
program. In this connection, each game played or each unit
20   of time played generates one or more reward points. Players
may also accumulate points for achieving certain results in
a game, such as, for example, one reward point per each
million scored points in a particular game. The reward point
redemption instructions associated with a given game program
25   26 can reside in the Software Control Block 706 or the
Secure Software Component 710 for that program in the
insecure data source 704, or be permanently stored in the
meter's non-volatile memory 506. During play, the meter 502
tracks reward points and stores such points in a reward
30   point database in its non-volatile memory 506. To redeem
reward points, a player requests a Reward Point Redemption
function, which directs the meter 502 to generate an
Authenticatable Point Redemption Message APRM, which
includes ID_Meter or the secret computer or software ID
35   SSCID, player PIN or other identifier, number of points for

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c which redemption is requested, and the current Date/Time. This APRM may be encrypted with the SSCID and/or with the meter's secret key SK_Meter. The central computer 12 reads and authenticates the Authenticatable Point Redemption Message APRM and increments the player's reward point

5 balance by the appropriate number. These reward points may be subsequently utilized to purchase prizes or gifts, which purchases may be made in combination with additional payment if desired.

In an exemplary embodiment, the credits used in

10 the pay-per-use process may be transferred between game computers 14 (i.e., from a source game computer 14 to a destination game computer 14) such that a player can bring credits to play on another player's game computer 14. This may be implemented by requesting a Credit Transfer function

15 associated with the meter 502. The meter 502 prompts the player for a PIN, Account_Number, or other identifier to verify the player's identity. If this response is consistent with the data stored in the memory of the meter 502, the process proceeds with the player entering the game computer

20 ID number or a public key PK_Comp of the destination game computer 14, and/or the ID_Meter or public key PK_Meter of the meter 502 associated with the destination game computer 14. The source meter 502 then generates an Authenticatable Transfer Message ATRM incorporating the Limit_Time or

25 Limit_Cost for use on the destination game computer 14/meter 502 system, a PIN or other player identifier, the game computer ID number of the destination game computer 14, and the current Date/Time from the clock 512 associated with the source meter 502 or the clock 36 of the source game computer

30 14. This message may be encrypted or signed with the public key PK_Comp of the destination game computer 14 or the public key PK_Meter of the destination meter 502 associated with the destination computer 14. The source meter 502 then decrements the credit balance in its non-volatile memory 506

35 by the corresponding amount requested. The player writes

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down or prints out the Authenticatable Transfer Message ATRM (or downloads it to portable data memory media such as a floppy disk or PCMCIA card) and inputs the same into the destination meter 502 associated with the destination game computer 14. The destination meter 502 associated with the destination game computer 14 reads and authenticates the Authenticatable Transfer Message ATRM in accordance with the cryptographic protocols described above, such as, for example, by decrypting the ATRM with the destination meter's private key PR_Meter (if the ATRM was encrypted with the destination meter's public key PK_Meter) or with the destination computer's private key PRK_Comp (if the ATRM was encrypted with the destination computer's public key PK_Comp), and stores the Date/Time in memory, and compares the Date/Time with previous messages to determine whether that Authenticatable Transfer Message ATRM has been used previously. If the Authenticatable Transfer Message is authenticated and the credit transfer authorized, the transfer credits are added to a "guest account" generated by the destination meter 502 associated with the destination game computer 14. The player uses these credits by entering his or her PIN or other identifier incorporated in the Authenticatable Transfer Message ATRM. The meter 502 then enables game play as described above. If a credit balance remains after play, the destination meter 502 generates an Authenticatable Balance Return Message ABRM, which incorporates the number of credits returned Limit_Cost and/or Limit_Time, the player PIN, source game computer ID number or source ID_Meter, and the current Date/Time. The ABRM may be signed with the private key PRK_Meter of the meter 502 associated with the destination game computer 14, or the private key PRK_Comp associated with the destination game computer 14. Contemporaneously, the guest account is deleted. The player enters the Authenticatable Balance Return Message ABRM into the source game computer 14 or meter 502 associated with the source game computer 14, and,

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if the meter 502 authenticates the ABRM, by, for example, decrypting the Authenticatable Balance Return Message ABRM with the public key PRK_Meter or PRK_Comp of the destination game computer, it adds the credits to the player's credit balance in the source meter 502.

5              Players may set up guest accounts on other player's computers via the central computer 12. In this connection, a player requests a guest account on another's game computer 14. The meter 502 displays an Authenticatable Guest Message AGM on the destination game computer 14, which

10       message includes the SSCID of the destination game computer 14, a player PIN or other identifier, and the current Date/Time. This message may be encrypted with a public key PK_CC from the central computer 12. The player then calls the central computer 12, and is prompted to enter the

15       Authenticatable Guest Message AGM, accompanied by the number of credits requested and a credit card number or some other instructions to arrange for payment. The central computer 12 authenticates the Authenticatable Guest Message AGM with the encryption/decryption module 52, and generates in return an

20       Authenticatable Guest Credit Message AGCM, which includes the destination game computer SSCID, the number of credits requested, and the current date/time. This message may be encrypted with the SSCID of the destination game computer 14. The meter 502 associated with the destination game

25       computer 14 then authenticates the Authenticatable Guest Credit Message AGCM, and adds the credits transferred to a guest account as described above.

              Although the foregoing embodiments utilize an meter which is either part of or associated with (e.g., by

30       way of a PCMCIA card and the like) the game computer 14, it is possible to incorporate the constituent components in a tamper-resistant game controller 800 as shown schematically in FIG. 28. One exemplary type of game controller is a joystick, in which player movements are transmitted to the

35       game computer 14 to control the movements of game

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characters. This allows for backward compatibility with existing game computers 14. A game controller 800 includes the meter components described in the foregoing, plus the additional hardware to enable input controls such as joysticks and buttons which communicate via an input interface associated with the game computer 14 as is well known in the art. The game controller 504 also includes a video input interface for receiving NTSC (PAL in Europe) video signals. A video output interface communicates the video signal to a TV. The game controller contains a program in memory for recognizing data from the video screen to derive the outcome information to generate Authenticatable Outcome Messages as discussed at length above. The game controller memory also stores a unique controller secret serial number CSSN, a random number generator program, and the meter program instructions. As described above, the encryption/decryption functions implemented by the encryption/decryption module 28, are part of the meter in this embodiment. In this regard, the controller memory stores associated public and private encryption keys, and a public encryption key associated with the hardware manufacturer. The game software 15 contains or can read from another source, the public key of the controller 800, and the code used to generate random numbers. This code is obfuscated in order to render the operation of interposing devices, such as the above-described GAME GENIE, more problematic. In an exemplary scenario, a player purchases an Authenticatable Unlock Message AUM as described above, and enters it into the game controller 800. The game controller CPU under direction of the random number generator program generates a random number R1 and an Authenticatable Play Enable Message APEM authorizing play. This APEM includes the controller secret serial number CSSN, and may be encrypted with the private key of the game controller. The APEM is then communicated to the CPU 27 of the game computer 14 or to the descrambler 500 as described

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above. The game controller 800 also "signs" the public key of the game controller and the game controller secret serial number CSSN with the private key of the hardware manufacturer. The latter is known as a public key certificate. This enables the game computer 14 to authenticate the public key certificate and thus the game controller 504. Thus, the game computer 14 is able to verify that the controller secret serial number CSSN contained in the Authenticatable Play Enable Message APEM is correct for that game controller 504. The game computer 14 then generates a second random number R2, which is communicated to the game controller 504 along with random number R1. The game controller 504 confirms that this R1 matches the R1 previously created and included in the Authenticatable Play Enable Message APEM, and encrypts R1 and R2 with the private key of the game controller 504 into a response play enable message RPEM. The RPEM is read by the game computer 14 and decrypted with the public key of the game controller 504. R1 and R2 are now compared with the prior generated values. If they match, the game controller 504 is authenticated and it may be used to enable game play in accordance with the foregoing protocols.

The present invention has been shown and described in what are considered to be the most practical and preferred embodiments. It is anticipated however that departures may be made therefrom and that obvious modifications will be implemented by persons skilled in the art.

Glossary:

Authentication: The process whereby the identity of the sender and/or integrity of a message is verified.

Authenticatable Balance Return Message (ABRM): An authenticatable message generated by the destination game

computer which allows a player to return unused credits from a guest account back to the source game computer. The message may contain the secret serial number of the source game computer, the number of credits to return, the player PIN, and the current date/time.

5

Authenticatable Challenge Message (ACM): A message used in the challenge/response protocol that instructs the game software to generate an encrypted response message which enables the challenge/response protocol and which may also

10    verify that the player is in possession of, or obtained the game outcome from the game software which enabled the player to play the game that resulted in the outcome for which the player seeks registration.

15    Authenticatable credit authorization message (ACAM): An authenticatable message generated by the central computer which is used to supply the player with additional credits. The message may contain the secret serial number, the number of credits requested, PIN, and a random number or sequence

20    number to ensure message freshness. The secret serial number ensures that the credits received can only be used in one game computer.

Authenticatable Credit Request Message (ACRM): An

25    authenticatable message generated by the game computer as a request for additional credits from the central computer to be used in the pay-per-use system. The message may contain the secret serial number, the number of credits requested, PIN, a sequence number, or a random number. The random

30    number or sequence number are used to ensure message freshness. The secret serial number ensures that the returning ACAM can not be used in multiple game computers.

Authenticatable Flat Rate Request Message (AFRRM): An

35    authenticatable message generated by the game computer,

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which provides a player for unlimited game play over a specified time period. The message may contain the SSCID, the period of time requested, the player PIN or other identifier, and the current date/time.

5      Authenticatable Flat Rate Message (AFRM): An authenticatable message generated by the central computer in response to the AFRRM. The message may contain the SSCID, the period of time requested, the player PIN or other identifier, and the current date/time.

10

Authenticatable Guest Message (AGM): This authenticatable message is generated by the game computer and allows a player to set up a guest account without bringing credits from the source computer. The message may contain the secret

15     serial number of the destination game computer, PIN or other player identification, and the current date/time.

Authenticatable Guest Credit Message (AGCM): This authenticatable message is created by the central computer

20     in response to an AGM. The message may contain the secret serial number of the destination game computer, the number of credits requested, and the current date/time.

Authenticatable Outcome Message (AOM): An authenticatable

25     representation of the game outcome (i.e., score, time to completion, and/or play-related data, etc.) which may be based upon and include at least one of the following, the software ID, a hash value of the game software, a unique attribute of the memory media in which the game software

30     resides, a player PIN, etc.

Authenticatable Play Enable Message (APEM): An authenticatable message communicated to the CPU of the game computer, which authorizes game play. It may contain the

35     SSCID, a random number, or an identifying source of the

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APEM.

Authenticatable Point Redemption Message (APRM): An authenticatable message generated by the game computer, which allows a player to transfer reward points to the central computer. The message may contain the SSCID, the number of points received, the player PIN or other identifier, and the current date/time.

Authenticatable Response Message (ARM): An authenticatable message utilized in the challenge/response protocol, and which may also verify that the player is in possession of, or obtained the game outcome from the game software which enabled the player to play the game that resulted in the outcome for which the player seeks registration.

Authenticatable Start Message (ASTM): An authenticatable message generated by the central computer which enables tournament play to begin. The message may include instructions for the game software to generate specified or random initialization parameters for that tournament game. The message may also contain information regarding encryption keys to be used in the encryption process and/or handicapping parameters.

Authenticatable Timing Message (ATM): An authenticatable message representing the time to completion for a race of skill which may include at least one of the following, the software ID, a hash value of the game software, a unique attribute of the memory media in which the game software resides, player PIN, etc.

Authenticatable Transfer Message (ATRM): This message is used by players to transfer credit balances from one game computer to another. The message may contain the secret serial number, PIN or other player identifier, current

° date/time, and the game computer ID from the destination game computer. The ID from the destination computer prevents a player from using an ATM in multiple game computers.

5 Authenticatable Unlock Request Message (AURM): An authenticatable message generated by the game computer, which allows a player to request an extension of his current end date. The message may contain the SSCID of the game computer, the requested end date, and a random number.

10 Authenticatable Unlock Message (AUM): This authenticatable message is generated by the central computer in response to a request for a new end date. The message may contain the SSCID of the game computer, the new end date, and the random number received in the AURM

15

Authenticated Outcome Confirmation Message (AOCM): The authenticated game outcome (i.e., score, time to completion, and/or any play-related data).

20 Biometrics: The processes and procedures used to uniquely identify a given individual via an independent measurement system. (e.g. a fingerprint reader, voice recognition system, retinal scanner and the like.)

25 Broadcast Start Message (BSTM): A start message that is broadcast to all players at the same time over a mass communications channel, e.g., television, radio and the like.

30 Central authority: A system utilizing a central computer applying cryptographic protocols, which ensures that the manner in which games are played is scrupulously fair and unbiased, and that the outcomes of such games are reported accurately, i.e., there is no undetected lying or cheating.

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Central computer: A computer or network of computers which serve as the official tournament manager and/or the authenticator of scores. The central computer may or may not have some or all of the game software residing on it. The central computer is generally accessed via a telephone network from remote locations where the game computers are disposed. The telephone network may include interactive voice response units (IVRUs) for prompting the player to enter information through a telephone keypad, or the game computers may communicate directly through a modem.

Central scoreboard: A centralized database where all certified scores are maintained. This information could be made available via the Internet or major online providers, downloaded to game computers, or by mail or telephone.

Certified outcome: A game score, time to completion and/or any play-related data which has been authenticated by the central authority as having been achieved legitimately (fairly) and reported accurately.

Challenge/response protocol: A process by which a central computer exchanges messages with the game computer in order to authenticate the outcome message using cryptographic protocols, and may also prove that the player is in possession of, or obtained the game outcome from the game software which enabled the player to play the game that resulted in the outcome which the player submits for certification.

Cheat codes: When entered into the game software, these codes allow a player special benefits such as unlimited weapons or an infinite number of lives. Typically used by game programmers to test their software. Also referred to as "God codes."

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Check digits: Digits appended to a message which are generated as a simple function of the original message. The receiver of the message repeats the function. If the result does not match the check digits, a transmission error has occurred.

Classic tournament: . A non-electronic tournament. Classic tournaments usually contain four attributes: 1) They are held at a specific time; 2) They are held at a specific location; 3) They are conducted under a set of rules which apply equally to all contestants; and 4) They are held under the supervision of one or more judges and/or a sanctioning authority.

Compression: A protocol which utilizes an algorithm to reduce a long string of data to a shorter string while retaining all or most of the information in the original long string.

Controller secret serial number (CSSN): A number which uniquely identifies the game controller. This number may be burned into the ROM chip(s) of the controller.

Credits: Payment units used in a pay-per-use system. Players purchase credits from the central computer. Unused credits are stored in the meter of the game computer.

Cryptographic protocol: A protocol whose purpose, for the present invention, allows for one or more of the following:

1) outcome legitimacy, i.e., the player did not simply invent an outcome, it was the result of playing a specific game.

2) outcome paternity, i.e., the outcome was

generated by a specific game, and/or by a specific game computer and /or software package.

3) outcome integrity, i.e., the outcome has not been tampered with or modified in any manner.

4) software integrity, i.e., the game software has not been tampered with or modified in any manner.

5) tournament validity, i.e., the outcome was generated as a result of tournament and not practice play.

6) one-time usage, i.e., an Authenticatable Start Message may only be used once.

7) transmission integrity, i.e., the outcome represented in an encrypted outcome message cannot be altered during transmission, either accidentally or intentionally, without detection of such alteration.

8) non-repudiation, i.e., does not allow the player who registered a score with the central computer to subsequently deny having done so.

Dedicated game system: A game console such as those manufactured by Sega or Nintendo. Unlike a PC, their primary use is to play games.

Destination game computer: When setting up a guest account, this is the game computer which is receiving the credit transfer from the source computer.

Digital signature: A sequence of bits based on both the message being signed and the signer's private key that the receiver can use to verify both the identity of the sender and the integrity of the message.

Division: Tournaments may contain different player groups such as beginner, intermediate and advanced, or groups delineated by geographic location.

5    Encryption: The process of describing a message in such a way as to hide its substance.

Encrypting key: Any data string used to encrypt a message in a cryptographic protocol.

10   Encryption/decryption module: A computer program, block of code, or hardware/firmware which encrypts, decrypts and authenticates messages and/or data.

End date: A date stored within the non-volatile memory of
15   the meter. When the current date exceeds this date in time, the game computer and/or game software is disabled. Alternatively, a descrambler device for descrambling a scrambled video signal is disabled.

20   Entry: Each separate opportunity to play in a tournament. Each entry may allow for multiple rounds of play. Some tournaments may allow players to enter multiple times.

Entry fee: The cost to purchase each entry. Players might
25   pay by giving their credit card number to the tournament central computer. A specific number of entry fees can be included at no extra cost with the purchase of game software.

30   External authentication device: A secure device in which the encryption/decryption module resides, e.g., the iPower card, available from National Semiconductor Corp.

External clock signal: A time signal generated by a third
35   party that is widely broadcast. Several government operated

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atomic clocks broadcast such signals throughout the United States, as does the Global Positioning System. This external clock signal may also be directed to a particular receiver if the signal is encrypted with that receiver's public key.

5    False score: A score which was obtained by altering the game software or by invoking "cheat codes."

Firmware: Programs stored in ROMs or in other devices which permanently keep their stored information.

10

Forged score: A counterfeit score which is used to try and deceive the central authority into believing that a fair game was played and a true score achieved when in fact the player made up the score or stole it from another player.

15

Game computer: The computer on which a game (or test) is being played. The game computer may be a video game console (e.g., Nintendo, Sega and the like), PC, PDA, coin-operated arcade machine, portable game units (e.g., GAME BOY, GAME

20   GEAR and the like), etc.

Game of skill: Any game whose outcome is predominantly determined by skill, either per game played or over a series of games, e.g., backgammon, bridge, poker, etc. Games of

25   skill may include components of luck such as, for example, the use of dice.

Game of chance: Any game where randomness is a key or dominant element in determining the outcome, or where the

30   outcome is determined largely by external forces beyond the control or significant influence of the player.

Game parameters: Attributes of a game such as the number of levels, difficulty or variability. These parameters can be

35   manipulated by the central computer and communicated to the

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game computer through the Authenticatable Start Message.

Game software: Code executed by the game computer which allows a player to play a game. The game software typically resides on a cartridge, CD-ROM, floppy disk, hard disk, etc.

Guest account: A temporary account for pay-per-use game play set up on another player's game computer. This account is managed by the game computer's meter.

Handicap device: Head-to-head games in which one player is clearly better can be handicapped by using initialization variables that provide the weaker player with more lives, more ammunition, and the like. The handicap device produces these initialization variables based on player ratings or titles.

Handicap values: A numerical method for equalizing two factors such as players of varying ability. A golf handicap, for example, might be four strokes.

Hardware inspection: The process by which a piece of hardware, such as a game cartridge, is physically inspected prior to a final official declaration. In a tournament where the grand prize is large enough, the winning player may be required to submit his or her game cartridge to prove that tamper-evident internal seals were not broken.

Hardware security: The use of specialized components or structures that provide a level of tamper-resistance and/or anti-counterfeiting to programs or results.

Hash function: A function that takes an input string (usually a series of digits) and converts ("hashes") it to a fixed size, usually smaller, output string. The object is to "fingerprint" the input string so that the resulting hash

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value is very likely to represent one and only one input string. Changing a single bit of the input string will result in a different hash value.

5 Hash value: The resulting string of data ("the fingerprint") after applying a hash function to an input string.

Instant prize: A prize which is earned for achieving a result without any consideration of what may be taking place in other related games or with other contestants. A player
10 in a golf tournament might be eligible to win $5,000 for hitting a hole-in-one on a certain hole without consideration to his or her ranking in the overall tournament.

15 Internal clock: A clock contained within a game or computer system.

IVRU's: Interactive Voice Response Units are computer systems that are tied into a telephone in such a manner that
20 a user may select prompts and enter data from a touch tone keypad or via voice commands recognized by the IVRU software.

Key card: A smart card which is used for the secure
25 generation of encryption keys. Each card may be unique, and messages encrypted using the keys in the card are decryptable by the central computer.

League play: Team play in which there are a series of
30 regularly scheduled matches.

Memory media signature: A unique readable set of data which is based upon an inherent property of a particular memory media and which cannot be easily duplicated.

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° Non-volatile memory: Data storage media including ROM, hard disks, floppy disks, optical disks, and the like, which do not require any power supply to maintain the memory contents.

5 Off-line: When a computer is not connected to (e.g. via modem) or receiving an electromagnetic external signal of any kind (e.g. RF signal) from another computer or outside source.

10 One-way function: A function that is relatively easy to compute but significantly harder to reverse or undo. For example, it is easy to multiply two X-digit numbers and create a Y-digit result. It is very hard to take an Y-digit number and figure out which two X-digit numbers were 15 multiplied together to create the Y-digit number.

One-way hash function: A hash function that is also a one-way function. It is easy to compute a hash value from an input string but it is hard to generate a string that hashes 20 to a particular value. A One-way hash function is also known as a compression function, contraction function, message digest, fingerprint, cryptographic checksum, data integrity check (DIC), manipulation detection code (MDC), message authentication code (MAC), and data authentication 25 code (DAC).

One-way hash with key: One-way hash functions that require the use of both an input string and a specific key string. (The "key" is an additional input string which may be 30 secret.) Only someone with the key can calculate the hash value, i.e., the hash value is encrypted with the key.

On-line: When a computer is connected to or receiving a signal from another computer. Typical examples include PCS 35 on a local area network, PCS connected to the Internet using

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a modem and phone line, or cellular phones connected to the central phone switch over a wireless network.

On-line proctor: An on-line connection to a website or service whose purpose is to continuously or intermittently verify that a tournament game is proceeding in a manner consistent with the game's rules. This might involve temporary connections to verify that the software code governing the game has not been altered. It might also pose random simple questions to the player (e.g. what is your middle name) that would prevent an automated play system from being used.

Outcomes: A score, time to completion, and/or any play-related data that was the result of all or part of playing the game. The play related data may include special statistical information as determined by the game software, or the play related data can be used by the central computer to compile statistical information. Alternatively, an outcome may consist of all the game data for the entire game, i.e., the entire game is recorded in memory, including all of the player's actions, responses, moves and the like.

Outcome message: Outcome data exchanged between a game computer and the central computer.

Public-key certificate: A public key signed by a trusted authority.

Premiums: Non-cash prizes, e.g., tee shirts, coffee mugs, posters, certificates, etc.

Pre-paid tournament entries: Tournament entries paid in advance of the tournament start date.
Prize: A product or cash reward associated with the outcome of a tournament. Typically, players earn prizes in relation

to their performance in the tournament. Cash prizes can be paid via check, electronic fund transfer, digital cash or credit card. Prizes may also take the form of credits toward future game play, or may represent non-material rewards such as recognition.

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Qualification points: Points earned toward a given rank. Bridge players, for example, receive master points for tournament wins.

10    Ranking: The hierarchical position of a certified score (#1, #225, etc.). The hierarchy can be based on all contestants or limited to certain agreed groups or limitations (e.g. #4 in the state, #6 in a high school, etc.).

15

Rating: A value assigned to a player based on one or more certified scores, often achieved over a period of time. A chess rating is expressed as a 3 or 4-digit number such as 1,825. Ratings are based on achievement, not on position

20    relative to others (rankings). Thus, two players could each have the same chess rating.

Sanctioning authority: The authority responsible for overseeing the policies and procedures used in connection

25    with a tournament.

Save/resume code: A code which allows a player to temporarily stop a game, freezing the action until the resume code is entered into the game software.

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Secure perimeter: A defined physical area of hardware which is tamper-resistant and/or temper-evident, in which resides data or algorithms whose characteristics must not be alterable in order for a system to remain secure.

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"Self-starter" tournament: A tournament where the element of when the player competes is controlled by the player, though a permissible window of time may be set forth by the tournament director. A tournament to solve a puzzle where the solution is expected to take several weeks, might allow for players to start when they wanted so long as the player's start time is after a predetermined start date for the tournament.

Software ID number: A unique ID number incorporated into the game software which uniquely identifies the particular copy of the game software and which is difficult to alter in an undetectable manner.

Software registration process: A process by which the player sends personal information and a serial number SSN identifying a copy of the game software back to the central authority. Each game software copy may utilize a registration card having a special hologram and/or serial number SSN associated therewith which is difficult to forge.

Software security: The use of cryptographic techniques such as hash functions to ensure the integrity of the game software.

Software serial number SSN: A readily ascertainable (i.e., known) identification number associated with each copy of the game software. Not to be confused with the software ID contained within the game software and which is not known to or easily accessible by the player.

Source game computer: When setting up a guest account, this is the game computer which is sending the credits to the destination computer.

Sprites: The video representation of game characters.

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Players maneuver sprites with the game controller.

Start message: A message transmitted from the central computer to the game computer that activates a tournament game.

Submission: The process by which a player transmits a game outcome to the central computer.

Subscription fees: Monthly or yearly charges which allow a player a certain number of tournament entries.

Tamper-resistant: Hardware or software which is difficult to modify or alter from its intended purpose. In some cases, attempts to alter such hardware or software will render the hardware or software inoperable. In other cases, tampering is detectable by cryptographic tests.

Tamper-evident: Hardware or software which, upon inspection or interrogation shows evidence of any attempt or success at the modification or alteration of its intended purpose or stored data.

Tie-breaker: A secondary result other than the final score which is used to rank players of equal scores in lieu of a head-to-head playoff. For example, a computerized golf tournament might decide the winner based on the score, and, if scores are tied, then the tie is broken based on play-related information such as the fewest putts taken, and if the players are still tied, then based on the distance of the longest drive. Tie-breaks can also be based on, for example, the number of keystrokes or clicks of a mouse.

Time-stamp: A secure timing protocol for attaching the current time and/or date to a message. A time-stamp may be done with a secure device such as the central computer or

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via a trusted third party such as a digital time stamping service.

Title: Ratings within a given range qualify their holders to a "rank" or honorific title based on predetermined ranges. Thus a chess "expert" is rated between 2,000 and 2,200 and a black belt in karate is someone who has achieved certain measured standards of proficiency. The achievement of a certain title is not necessarily based on numerical scoring, but rather on certain play-related data such as number of levels completed or number of opponents defeated and the like.

Token: A tamper-resistant and/or tamper-evident portable device (e.g., an iPower card) used for one or more of the following:

1) storing of secret or private keys.

2) storing random digits.

3) encrypting and/or decrypting messages.

4) signing and/or verifying messages.

Tournament: Any contest where players, singly or in teams, compete for prizes and/or rankings.

Tournament ID number: A number uniquely identifying the tournament.

Transmission error check module: A computer program or block of code which examines the check digits on incoming messages to ensure that no transmission errors have occurred.

Types of tournaments-There are many different types of

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tournaments. The following represent some of the more common varieties:

In a scoring tournament, a player plays against the game (and/or a clock) and the highest score wins. Golf, bowling or duplicate bridge are classic scoring tournaments.

In a head-to-head tournament, players compete against each other instead of against the game. Boxing and tennis are classic head-to-head tournaments. These tournaments are interpersonal in nature.

In a puzzle tournament, players compete to solve a puzzle, sometime ranked by time, other times, simply in a race to be first. A crossword puzzle tournament might be a good example of this.

Talent competitions are another type of tournament. Unlike scoring tournaments which usually do not involve subjective elements, a talent competition utilizes judges to evaluate performance and award scores based on subjective elements. A gymnastics tournament is an example.

Finish-line races, are a form of head-to-head tournaments whereby the fastest time as measured against a field of competitors is the determinant of winning. A car race or 100 yard dash is won by the person who finishes first, regardless of the time involved.

Forecasting competitions are those that involve the comparative accuracy of a series of predictions, such as handicapping a football season or predicting a variety of financial indicators at some future time. Lottery games such as Lotto may be thought of as a non-skill forecasting tournament.

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Note that all types of tournaments can either be single-player or multi-player (teams).

Volatile memory: memory media such as RAM, in which all stored data is lost if power is interrupted.

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Website: Any computer connected to the Internet which is capable of being accessed via the World Wide Web.

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## Industrial Applicability

In view of the foregoing detailed description, it is evident that the instant invention may be used to create one or more of the following systems, among others:

5      - a system for enabling geographically dispersed tournaments for computer generated games in which players can participate from virtually any location where they have access to a game computer (e.g., at home), without the need for an on-line connection between the game computer and a

10     central computer while the game is being played;

- a system for certifying the outcome of a computer generated game on a game computer and for ranking and rating the player based on that outcome or an aggregation of

15     outcomes, with respect to other players of the game, by authenticating the outcome(s) of the game utilizing a central computer, either in connection with a given tournament or independent thereof, thereby eliminating the need for a trusted third party to be present at the

20     tournament site or to be on-line to ensure that the outcomes were legitimately achieved and accurately reported;

- a system that enables a test taker of a computer administered test on a game computer, where the test is not

25     provided on-line, the test software residing or associated with the game computer, to have his or her test score certified with a central computer and to obtain a ranking and rating with respect to other test-takers;

30     - a system for certifying outcomes of computer generated games played on game computers, and for ranking and rating the players of such games based on their outcomes or an aggregation of their outcomes, with respect to other players of the games, with a central computer having a database

35     storing a unique attribute or identifier for each game

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computer or software, by generating authenticatable messages on the game computer that represent the players' game outcomes and the unique attribute or identifier associated with the particular game software or the game computer, and authenticating the authenticatable message at the central computer using cryptographic protocols;

- a system for providing cash prizes or other awards or tokens of recognition for players in accordance with their certified ranking and/or rating as described above;

- a system for certifying times to completion for races of skill played on game computers which start at designated times, either in connection with a given tournament or independent thereof, where the first participant to complete the game and have his or her time of completion certified by the central computer is declared the winner, and for enabling the participants to be ranked and rated with respect to each other;

- a system for races of skill tournaments, where the start times of the games are variable and players are ranked by the length of time it takes to finish playing the games as determined by a clock associated with the game computer or an external clock signal broadcast over a mass communications means, where the time is authenticated at the central computer and the player finishing a given game in the shortest amount of time is declared the winner;

- a system for rating/ranking players in tournaments engaged in races of skill as described above, where the players obtain scores for the games where these scores are adjusted by the amount of time it took to complete the games and/or any other play conditions, at the central computer;

- a system for rating/ranking players in tournaments where

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groups of players form teams and the team scores are certified and ranked at the central computer;

- a system in which players engage in tournaments on game computers, where a start message which enables tournament play contains variables which are read by the game computers and direct the game programs to set game parameters based on player's individual ratings or other parameters, with certain specified attributes or other programmed characteristics, e.g., difficulty, variability, randomness, etc;

- a system in which players engage in tournaments on game computers where the players decide when they want to enter the tournaments and play;

- a system in which players engage in tournaments on game computers and where hardware security and/or cryptographic protocols are utilized to ensure the fairness and integrity of the tournament:

- a tournament system using cryptographic and other protocols, where a trusted third party is not required to prevent undetected player substitution:

- a system where the outcomes of computer games of chance are submitted to a central authority and certified using cryptographic and other protocols;

- a system in which players of video games having different ratings/skill levels may play head-to-head matches where the playing conditions during the game are equalized in response to handicap codes;

- a system wherein a computer generated result or outcome obtained on a computer is incorporated into an

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Authenticatable Outcome Message by the computer, and may be subsequently authenticated on the computer with cryptographic protocols;

5      - a system in which a computer generated result or outcome obtained on any computer in the system is incorporated into an Authenticatable Outcome Message by that computer, and may be subsequently authenticated on any other computer in the system with cryptographic protocols;

10     - a system in which all data in connection with recreating a game played on a game computer may be stored on removable data memory media in an authenticatable format and subsequently used to generate a replay of the game on any game computer in the system by authenticating the data using
15     cryptographic protocols;

- a system in which a device placed between a game computer and a TV, reads the data in a video output signal to obtain an outcome for the game from the video output signal, and
20     incorporates the outcome into an Authenticatable Outcome Message;

- a system in which a device compatible with a VCR is placed between a game computer and a TV, reads the data in the
25     video output signal, converts the data to digital format, makes the data authenticatable using cryptographic protocols, and stores the authenticatable data in data memory media for subsequent authentication and play back;

30     - a pay-per-use system for enabling video arcade type play on home game computers;

- a pay-per-use system for enabling time-dependent disablement with cryptographic protocols of game computers
35     and/or game software; and

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- a multi-functional game controller for implementing the foregoing with existing game console-type game computers.

In general, the present application presents a system for authenticating the outcomes of computer generated games played on game computers, and for certifying those outcomes as being accurately reported and fairly achieved. The system provides for such certification in connection with tournaments or independent thereof. The system may include, in one embodiment, a plurality of game computers, where each game computer includes associated memory and a processor for executing programs from its associated memory. The term "associated memory" is intended to include the internal read only memory ROM and read-write memory RAM of the game computer, as well as external devices such as hard disk drives, CD-ROM drives, floppy disk drives, game cartridges and the like. This memory is generally insecure, and may also be referred to as an insecure data source. The game computer contains game software including at least one game program that is executed by the processor to enable a player to play a game on the game computer. The games may be games of skill, races of skill, games of chance, predictions on future events of which the outcome is uncertain, and the like. In a game of skill, the game has an outcome as a result of game play, where the outcome is defined as the entire set of results of the game, including a score, time to completion, all data relating to the game itself, and any play related data. In the present invention, the outcome of the game is incorporated into an Authenticatable Outcome Message AOM that may be subsequently authenticated on the same game computer itself, any other game computer, or by a central computer. In some embodiments described herein, the authentication process not only authenticates but also certifies the outcome as being accurately reported and fairly achieved.

In general, the present application also provides

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for an authentication means for generating and authenticating authenticatable messages which is operatively associated with the processor of the game computer. The authentication means comprises what is referred to herein as an encryption/decryption module that utilizes cryptographic protocols. The encryption/decryption module may be part of the game software disposed in the associated memory of the game computer, or dedicated firmware disposed within the game computer. Preferably, however, the encryption/decryption module resides within a secure perimeter or security token. The Authenticatable Outcome Message may include data that reveals if the game software has been tampered with by the player. This data is also generated, checked and verified using cryptographic protocols. An authenticated outcome that is determined to have been achieved without cheating the game software or the game computer is certified. The Authenticatable Outcome Message generated by the encryption/decryption module may be subsequently authenticated on the same game computer, on any other game computer with an encryption/decryption module, or by a central authority on a central computer.

The central computer of the present application may include an associated memory, a processor for executing programs from the central computer associated memory, and central computer authentication means operatively associated with the processor of said central computer for generating and authenticating authenticatable messages. The central computer authentication means are operable to authenticate Authenticatable Outcome Messages to authenticate game outcomes in response to authentication requests. By checking data appended to the outcome, the central computer can ascertain whether a player obtained the outcome by "cheating" the game software. The central computer may contain a plurality of relational databases for both certifying scores and managing tournaments.

Where a central computer is used to certify

outcomes and manage tournaments~, communications between the
game computers and the central computer may be transmitted
via a telephone network. The telephone network may enable
communication with live operators, but is preferably coupled
to Interactive Voice Response Units IVRUs. The IVRUs are
5    employed to prompt players to enter required information in
connection with registering for tournaments and/or for
submitting outcomes embodied in Authenticatable Outcome
Messages for certification. Alternatively, the game
computers may establish an on-line connection to the central
10   computer for the purpose of transmitting registration data
and Authenticatable Outcome Messages. The on-line connection
may take place over a data network including commercial
on-line service providers, Internet, World Wide Web,
bulletin board systems or over RF, cable TV, satellite links
15   and the like.

Another aspect of the invention provides for
pay-per-use of the game computer or game programs that are
executed on the game computer. The pay-per-use system
20   includes a meter that communicates with the game computer,
and operates to enable operation of the game computer or
execution of game programs upon authorization from the
central computer. The meter is a secure device, a computer
having hardware disposed within a secure perimeter, capable
25   of generating and authenticating authenticatable messages as
described above. In a preferred embodiment, the meter
controls operation of the game computer and/or game programs
using cryptographic protocols.
In the system of the present application, the
30   operating system program of the game computer and game
programs, are referred to as metered programs. Each metered
program is comprised of a Software Control Block, an
Insecure Software Component, and a Secure Software
Component. In a first embodiment, the entire metered program
35   resides in an insecure data source associated with the game

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computer, such as a hard disk or the like. The Secure
Software Component is a cryptographically secure set of
software instructions, that are decrypted by the meter and
executed on the meter to produce at least one output
parameter upon which the Insecure Software Component
5   depends, in order to execute the latter on the game
computer. The Software Control Block contains information
about the metered program that identifies it to the meter,
and, in some embodiments, enables the meter to calculate
costs for running that program. The meter decrypts and
10   executes the Secure Software Component as long as it has
authorization from the central computer, in the form of a
time or cost limit.

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WE CLAIM:

1. A computer device, comprising:

a memory device having encoding control code embodied therein; and

a processor disposed in communication with said memory device, said processor configured to process said encoding control code in conjunction with a computer game outcome to generate an encoded message corresponding to said computer game outcome and to transmit said encoded message to a human-readable output device.

2. The computer device of claim 1 wherein said human-readable output device is a display device.

3. The computer device of claim 1 wherein said memory device further contains game program execution code embodied therein, and wherein said processor executes said program execution code to generate said computer game outcome.

4. he computer device of claim 1 wherein said encoding control code comprises encryption protocol code.

5. The computer device of claim 1 wherein said processor is a first processor, and further comprising a second processor configured to generate said computer game outcome.

6. The computer device of claim 69 wherein said first processor is a secure processor.

7. The computer device of claim 1 further comprising a module containing said processor and said memory device.

8. The computer device of claim 7 wherein said

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module is selected from the group consisting of (a) a tamper-evident module and (b) a tamper-resistant module.

9.    The computer device of claim 7 wherein said module is a plug-in module having an electrical connector configured to plug in a computer game selected from the group consisting of (a) a dedicated computer game and (b) a personal computer.

10.    The computer device of claim 1 wherein said processor is further configured to periodically check blocks of computer game program execution code.

11.    The computer device of claim 1 wherein said processor is further configured to generate and include additional information in said encoded message, said additional information selected from the group consisting of (a) tamper-evidence information, (b) user identity information, (c) unique digital signature information, (d) global positioning information regarding a global position of said computer device, (e) a random number generated by a central computer, (f) a number corresponding to a time at which said outcome was generated, (g) a number which is incremented upon each successive outcome of said computer game, (h) an end parameter received from a central computer, (i) symmetric key information, (j) public key information, (k) hashing algorithm information and (l) metering information.

12.    The computer device of claim 11 wherein said unique digital signature message contains information selected from the group consisting of: a secret software identification message, a secret computer game identification message, a hash value corresponding to biometric user identity information, and a compressed value corresPonding to biometric user identity information.

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13.    The   computer   device   of   claim   1   further
comprising said display device.

14.    A computer device comprising:
a   memory   device   having   embodied   therein
decoding control code and an encoded message corresponding
to a computer game outcome; and
a   processor   disposed   in   communication   with
said   memory   device,   said   processor   configured   to   process
said decoding control code to decode said encoded message to
reveal said computer game outcome.

15.    The computer device of claim 14 wherein said
decoding control code contains cryptographic protocol code.

16.    The computer device of claim 14 wherein said
processor   is   further   configured   to   process   said   decoding
control   code   to   decode   said   encoded   message   to   reveal
information   selected   from   the   group   consisting   of   (a)
tamper-evidence information,   (b) user identity information,
(c)   unique   digital   signature   information,   (d)   global
positioning   information   regarding   a   global   position   of   said
computer   device,   (e)   a   random   number   generated   by   a   central
computer,   (f)   a   number   corresponding   to   a   time   at   which   said
outcome   was   generated,   (g)   a   number   which   is   incremented
upon   each   successive   outcome   of   said   computer   game,   (h)   an
end   parameter   received   from   a   central   computer,   (i)
symmetric   key   information,   (j)   public   key   information,   (k)
hashing algorithm information and (l) metering information.

17.    The   computer   device   of   claim   14   wherein   said
processor   is   further   configured   to   determine   whether   said
computer   game   outcome   is   fraudulent   based   on   information
revealed from said decoded message.

18.    The   computer   device   of   claim   14   wherein   said

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memory device further has embodied therein an identification message containing identity information regarding a player who generated said computer game outcome, and wherein said processor is configured to determine whether said computer game outcome is fraudulent based on said decoded message and said identification message.

19. The computer device of claim 14 wherein said memory device further has embodied therein registration information regarding a player who generated said computer game outcome.

20. The computer device of claim 14 wherein said processor is configured to generate and transmit an authorization signal to a meter in at least one computer game to authorize execution of said computer game.

21. The computer device of claim 14 wherein said memory device and said processor are configured to rank a plurality of computer game users based upon a plurality of computer game outcomes.

22. The computer device of claim 14 wherein said processor and said memory device are configured to maintain and transmit a plurality of encryption keys to a plurality of computer games for mutual authentication of computer game outcomes.

23. The computer device of claim 14 wherein said processor and said memory device are configured to generate, maintain and transmit a unique registration number for each of a plurality of computer games.

24. A method, comprising the steps of:
executing a computer game program to generate a computer game outcome;

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encoding the computer game outcome to generate an encoded message; and providing said encoded message to a user.

25.    The method of claim 24 further comprising the step of:

providing by said user said encoded message to a device configured for decoding said encoded message.

26.    The method of claim 25 wherein said step of providing said encoded message to a user comprises the step of displaying said encoded message to said user.

27.    A method, comprising the steps of: receiving from a user an encoded message corresponding to an outcome of a computer game; decoding said encoded message to retrieve said outcome; and storing for further use said decoded message.

28.    A method, comprising the steps of: receiving from a plurality of users a plurality of encoded messages, each of said encoded messages corresponding to an outcome of a computer game;

decoding said encoded messages to retrieve each outcome;

ranking said outcomes; and

ranking said plurality of users based on the ranking of outcomes.

FIG. 1A

FIG. 1B

**FIG. 2**



**FIG. 3**

23

| GAME PROGRAM | 26 |
| ENCRYPTION/DECRYPTION MODULE | 28 |
| TRANSMISSION ERROR CHECK MODULE | 30 |
| TIME/DATE MODULE | 33 |
| BIOMETRIC DATA | 35 |
| SECRET SOFTWARE ID | 32 |

15

STATISTICAL DATABASE — 31

115

ACOUSTIC COUPLER

TO/FROM SECURE PERIMETER 300 (FIG. 4C)

27

39

CPU

INTERFACE CARD

GPS

CLOCK

BIOMETRIC DEVICE

37

36    38

31

FIG. 4A

GAME
CARTRIDGE 21

MEMORY 23a

| 26 | GAME PROGRAM |
|---|---|
| 28 | ENCRYPTION/DECRYPTION MODULE |
| 30 | TRANSMISSION ERROR CHECK MODULE |
| 35 | BIOMETRIC DATA |
| 33 | TIME/DATE MODULE |
| 32 | SECRET SOFTWARE ID |

15

19

I/O

25

SOFTWARE
SERIAL NO. SSN

**FIG. 4B**

300

36'

| 302 | CPU | CLOCK |
|---|---|---|
| 304 | ROM | NON-VOLATILE MEMORY 313 |
| 28 | | I/O → TO CPU 27 |
| 308 | RAM | POWER |

312

310

**FIG. 4C**

FIG. 4D



FIG. 4E

**FIG. 4F**



**FIG. 4G**

GAME COMPUTER 14

| VIDEO OUTPUT |

TO TV 402

VCR 400

| FRONT END RECEIVING CIRCUITRY | 412

| READ/WRITE HEAD | 408

300'

| INTERFACE CIRCUITRY | 406

| A/D CONVERTER | 410

302 — | CPU | — | CLOCK |

36'

310

308

304    414

| POWER | — | RAM |    | ROM |    | EPROM |

# FIG. 4H

FIG. 5

GAME SOFTWARE 15

BLOCK 1     BLOCK 2     BLOCK N

SOFTWARE
INSTRUCTIONS

• • •

314     314     314

GAME COMPUTER 14

RAM

BLOCK 1     BLOCK 2     BLOCK N

SOFTWARE
INSTRUCTIONS

• • •

TO CPU 27

314     314     314

SECURE PERIMETER 300

302 — CPU   CLOCK 36'

304 — ROM   NON-VOLATILE MEMORY

313

28 — RAM   POWER

308    310

HASH ALGORITHMS
HASHES OF BLOCKS $h_1 \ldots h_n$
MASTER HASH $h_m$

(1) GAME 1
(2) GAME 2
    :
(N) GAME N

FIG. 6A

FIG. 6B

42

| GAME DATABASE | 44 |
| GAME ID | 64 |
| GAME NAME | 66 |
| TOURNAMENT ID | 68 |

| TOURNAMENT DATABASE | 46 |
| TOURNAMENT ID | 70 |
| TOURNAMENT DATE | 72 |
| DIVISIONS | 74 |
| PRIZES | 76 |
| SECRET SOFTWARE ID/SERIAL NO. | 78 |
| MEMORY MEDIA SIGNATURE | 80 |
| SOFTWARE HASH VALUE | 82 |
| ENTRY FEES | 84 |
| START MESSAGE | 86 |
| AUTHENTICATED OUTCOME MESSAGE/TIMING MESS. | 88 |
| QUALIFICATION POINTS | 90 |

| PLAYER INFO. DATABASE | 48 |
| PLAYER ID/BIOMETRIC DATA | 91 |
| SECRET SOFTWARE ID/SERIAL NO. | 92 |
| PLAYER NAME/ADDRESS/PHONE/AGE | 94 |
| TEAM ID | 96 |
| TOURNAMENTS PLAYED | 98 |
| QUALIFICATION POINTS EARNED | 100 |
| HANDICAPS | 101 |
| RANKINGS/RATINGS/TITLES | 103 |

| OUTCOME DATABASE | 50 |
| TOURNAMENT ID | 102 |
| SCORES/TIMES/RAKINGS/RATINGS/TITLES | 104 |
| PLAYERS/TEAMS | 106 |

| STATISTICS DATABASE | 51 |
| GAME ID | 51a |
| PLAYERS/TEAMS | 51b |
| STATISTICS | 51c |

| ENCRYPTION/DECRYPTION MODULE | 52 |

| TRANSMISSION ERROR CHECK MODULE | 54 |

| RATING/RANKING MODULE | 55 |

| TIME/DATE MODULE | 56 |

| OPERATING SYSTEM | 58 |

| CPU | |
| 60 | |

| CLOCK | |
62

COMMUNICATIONS
INTERFACE
TO IVRU

FIG. 7

108

GAME SOFTWARE PROVIDES
OPTION FOR TOURNAMENT
OR NON-TOURNAMENT GAME

TOURNAMENT     NON-TOURNAMENT

PLAY REGULAR
GAME

BIOMETRIC VERIFICATION

111

110

ENABLE TOURNAMENT OPTIONS     112

DISPLAY 800# AND TOURNAMENT
ID FOR PLAYER TO CALL     114

CONNECT TO CENTRAL COMPUTER     116

PROMPT PLAYER FOR TOURNAMENT
ID/GAME SOFTWARE SERIAL NO.     124

PROMPT PLAYER FOR PLAYER ID     118

ENTER TOURNAMENT ID/GAME
SOFTWARE SERIAL NO.     126

ENTER PLAYER ID     119

PROMPT PLAYER FOR DIVISION
# OF TOURNAMENT     128

120

DOES
PLAYER HAVE
ID
?     YES

130

ENTER DIVISION # OF
TOURNAMENT

NO

PLAYER REGISTRATION     122

| 8A |
| 8B |

FIG. 8

132

HAS
PLAYER PAID
A PRE-PAID TOURNAMENT
ENTRY FEE OR WERE A
NUMBER OF TOURNAMENT
ENTRIES INCLUDED IN
THE PURCHASE
OF THE GAME
SOFTWARE
?     YES

FIG. 8A

NO

PROMPT PLAYER TO
ENTER CREDIT CARD # ——134

ENTER CREDIT CARD # ——136

CENTRAL COMPUTER
GENERATES AUTHENTICATABLE ——138
START MESSAGE FOR THE
PLAYER'S TOURNAMENT GAME

PLAYER ENTERS AUTHENTICATABLE
START MESSAGE INTO THE ——140
GAME COMPUTER

GAME COMPUTER AUTHEN-
TICATES START MESSAGE ——142

PLAY GAME ——144

FIG. 8B

```
                   ┌─────────────────────┐
                   │     START GAME      │──146
                   └─────────────────────┘
                             │
                   ┌─────────────────────┐
                   │ GAME SOFTWARE TRACKS│
                   │   PLAY PARAMETERS   │
                   │  INCLUDING TIME TO  │──148
                   │   COMPLETION/PLAY   │
                   │  RELATED DATA, ETC. │
                   └─────────────────────┘
                             │
                   ┌─────────────────────┐
                   │GAME END - GENERATE A│──150
                   │ FINAL OUTCOME (I.E., A│
                   │  SCORE OR A TIME    │
                   │   TO COMPLETION     │
                   └─────────────────────┘
                             │
              152            ◇                    156
                 ╲      ╱  SUBMIT  ╲    NO    ┌──────────┐
                    ╱   OUTCOME      ╲───────▶│  RETURN  │
                    ╲       ?       ╱         └──────────┘
                       ╲         ╱
                          ◇
                          │ YES
                          ▼
                   ┌─────────────────────┐
                   │      GENERATE       │
                   │   AUTHENTICATABLE   │──154
                   │  OUTCOME MESSAGE    │
                   │        AOM          │
                   └─────────────────────┘
```

# FIG. 9

DISPLAY 800# FOR PLAYER TO CALL ─158

PROMPT PLAYER FOR TOURNAMENT ID ─160

ENTER TOURNAMENT ID ─162

CENTRAL COMPUTER ACCESSES DATABASE FOR THAT PARTICULAR TOURNAMENT ─164

PROMPT PLAYER FOR PLAYER ID, SOFTWARE SERIAL NO. SSN AND AUTHENTICATABLE OUTCOME MESSAGE AOM ─166

ENTER PLAYER ID, SOFTWARE SERIAL NO. SSN AND AUTHENTICATABLE OUTCOME MESSAGE AOM ─168

BIOMETRIC VERIFICATION ─169

CENTRAL COMPUTER AUTHENTICATES THE AUTHENTICATABLE OUTCOME MESSAGE AOM ─170

174─ IS OUTCOME AUTHENTIC ? ──NO──> REJECT OUTCOME ─176

YES

177─ CHECK SOFTWARE INTEGRITY ? ──YES──> CHECK SOFTWARE INTEGRITY ─179

NO

─180 TAMPERING ? ──YES

NO

ADD CERTIFIED OUTCOME TO DATABASE ─178

GENERATE AUTHENICATED OUTCOME CONFIRMATION MESSAGE AOCM ─181

GENERATE CURRENT PLAYER RANKING ─182

END TOURNAMENT ─183

SORT THROUGH FINAL PLAYER RANKINGS/ RATINGS TO DETERMINE WINNER (S) ─184

AWARD PRIZES ─185

FIG. 10A

PLACE FINGER OVER
SCANNING LENS OF
FINGERPRINT VERIFIER 31 — 169a

FINGERPRINT VERIFIER
DIGITIZES IMAGE — 169b

DIGITIZED IMAGE COMPRESSED
OR HASHED — 169c

CHECK VALUE IN
CENTRAL COMPUTER — 169d

PLAYER
ID
VERIFIED
? — 169e

NO → STOP

YES

CONTINUE

# FIG. 10B

CENTRAL COMPUTER
CHECKS DATABASE
TO DETERMINE WHETHER
THE SAME AOM WAS
PREVIOUSLY RECEIVED

186

WAS AOM
PREVIOUSLY
RECEIVED
?

188

NO

YES

GENERATE CHALLENGE
MESSAGE REPRESENTING
KEY $K_y$

190'

ENTER CHALLENGE
MESSAGE INTO GAME
COMPUTER

192'

GAME COMPUTER GENERATES
AUTHENTICATABLE RESPONSE
MESSAGE ARM WITH KEY $K_y$

194'

ENTER AUTHENTICATABLE
RESPONSE MESSAGE
ARM

196'

CENTRAL COMPUTER
AUTHENTICATES
AUTHENTICATABLE
RESPONSE MESSAGE
ARM

198

GENERATE CHALLENGE
MESSAGE REPRESENTING
KEY $K_x$

190

ENTER CHALLENGE
MESSAGE INTO GAME
COMPUTER

192

GAME COMPUTER GENERATES
AUTHENTICATABLE RESPONSE
MESSAGE ARM WITH KEY $K_x$

194

ENTER AUTHENTICATABLE
RESPONSE MESSAGE
ARM

196

FIG. 11

```
        ╭204
┌─────────────────────┐          ┌─────────────────────┐
│  BROADCAST START    │          │ GENERATE AUTHENTI-  │ ╭214
│     MESSAGE         │          │ CATABLE TIMING      │
└─────────────────────┘          │  MESSAGE ATM        │
        ╭206              │       └─────────────────────┘
┌─────────────────────┐  │                 │
│   ENTER START       │  │                 ▼
│     MESSAGE         │  │                 .
└─────────────────────┘  │                 .
        ╭208              │                 .
┌─────────────────────┐  │
│    START GAME       │  │
└─────────────────────┘  │
        ╭210              │
┌─────────────────────┐  │
│  GAME SOFTWARE      │  │
│ TRACKS ELAPSED TIME │  │
│  DURING RACE OF     │  │
│     SKILL           │  │
└─────────────────────┘  │
        ╭212              │
┌─────────────────────┐  │
│    CALCULATE        │  │
│  ELAPSED TIME       │  │
└─────────────────────┘──┘
```

FIG. 12

CALL 800# AND CONNECT TO CENTRAL COMPUTER _216_

REQUEST HEAD-TO-HEAD TOURNAMENT GAME _218_

PROMPT PLAYER A FOR PLAYER ID _220_

ENTER PLAYER ID _222_

PAY ENTRY FEE OR VERIFY PRE-PAID ENTRY _224_

CENTRAL COMPUTER SEARCHES FOR PLAYER B _226_

CENTRAL COMPUTER GENERATES AUTHENTICATABLE START MESSAGE ASTM _228_

PLAYERS A AND B ENTER START MESSAGE INTO RESPECTIVE GAME COMPUTERS _230_

PLAYERS A AND B ESTABLISH ON-LINE CONNECTION BETWEEN GAME COMPUTERS _232_

EACH GAME COMPUTER VERIFIES THAT THE SAME START MESSAGE WAS ENTERED _234_

PLAY GAME _236_

GENERATE AUTHENTICATABLE OUTCOME MESSAGE AOM INCORPORATING OUTCOME, PLAYER IDS, GAME SOFTWARE INTEGRITY DATA, ETC. _238_

FIG. 13

704

INSECURE DATA
SOURCE

503 — GAME PROGRAM — 26

GAME COMPUTER
14

503 — OPERATING SYSTEM
PROGRAM — 702

RECEIPT FILE — 712

ROM
508

ROM
510

CLOCK
512

BATT.
BACKUP

BATT.
BACKUP

CPU
504

POWER
514

I/O
515

NON-
VOLATILE
MEMORY
506

INPUT
CONTROLS
516

MODEM
520

502

TO/FROM
CENTRAL
COMPUTER 12

DISPLAY    518

FIG. 14

SOFTWARE CONTROL
BLOCK — 706

503 →

INSECURE SOFTWARE
COMPONENT — 708

SECURE SOFTWARE
COMPONENT — 710

FIG. 15

# FIG. 16

522 — | METER CALCULATES SK_METER |

524 — | METER CREATES INITIALIZATION MESSAGE |

526 — | METER ENCRYPTS INITIALIZATION MESSAGE WITH SK_METER |

528 — | CENTRAL COMPUTER RECOVERS SK_METER AND THE INITIALIZATION MESSAGE |

530 — | CENTRAL COMPUTER CREATES ACCOUNT NUMBER |

| CENTRAL COMPUTER CREATES INITIALIZATION RESPONSE MESSAGE | — 532

| CENTRAL COMPUTER ENCRYPTS INITIALIZATION RESPONSE MESSAGE UNDER SK_METER | — 534

| METER DECRYPTS INITIALIZATION RESPONSE MESSAGE AND STORES ACCOUNT NUMBER | — 536

| RUN SYNCHRONIZING CLOCK PROTOCOL | — 538

# FIG. 17

| | |
|---|---|
| **540** — METER READS SOFTWARE CONTROL BLOCK-EXTRACTS SOFTWARE ID | CENTRAL COMPUTER CREATES NEW PROGRAM RESPONSE MESSAGE — **548** |
| **542** — METER CREATES NEW PROGRAM MESSAGE | METER RECORDS KEY INFORMATION IN SOFTWARE_ KEY_ TABLE — **550** |
| **544** — SECURELY TRANSMIT NEW PROGRAM MESSAGE TO CENTRAL COMPUTER | METER RUNS UPDATING COST INFORMATION PROTOCOL — **552** |
| **546** — CENTRAL COMPUTER LOOKS UP SOFTWARE ID FOR KEY | |

# FIG. 18

554 — 
┌─────────────────────┐
│ METER GENERATES     │
│ AUTHORIZATION       │
│ REQUEST MESSAGE     │
└─────────────────────┘

556 — 
┌─────────────────────┐
│ CENTRAL COMPUTER    │
│ CALCULATES LIMIT_   │
│ TIME, LIMIT _ COST  │
└─────────────────────┘

558 — 
┌─────────────────────┐
│ CENTRAL COMPUTER    │
│ CREATES             │
│ AUTHORIZATION       │
│ RESPONSE MESSAGE    │
└─────────────────────┘

560 — 
┌───────────────────────────┐
│ METER VERIFIES            │
│ AUTHORIZATION             │
│ RESPONSE MESSAGE          │
│ AND COPIES NEW LIMIT_     │
│ TIME AND NEW              │
│ LIMIT_COST INTO           │
│ MEMORY                    │
└───────────────────────────┘

# FIG. 19

562 — 
```
METER CREATES COST
UPDATE REQUEST
MESSAGE FOR
SOFTWARE_ID
```

564 — 
```
CENTRAL COMPUTER
CREATES COST UPDATE
RESPONSE MESSAGE
FOR SOFTWARE_ID
```

566 — 
```
METER MERGES NEW
COST_TABLE
INFORMATION INTO
INTERNAL COST_TABLE
```

# FIG. 20

568 — METER CREATES TIME UPDATE REQUEST MESSAGE

↓

570 — CENTRAL COMPUTER READS METER'S DATE/TIME-REPLIES WITH TIME UPDATE MESSAGE

↓

572 — METER CHECKS SEQUENCE NUMBERS AND ENCRYPTION-NOTES TIME FROM TIME UPDATE MESSAGE-RETURNS TIME UPDATE ACKNOWLEDGMENT MESSAGE

→

CENTRAL COMPUTER COMPARES TIME BETWEEN WHEN IT SENT THE TIME UPDATE MESSAGE AND WHEN IT RECEIVED THE TIME UPDATE ACKNOWLEDGMENT MESSAGE-IT THEN SENDS A TIME UPDATE OK MESSAGE — 574

↓

METER UPDATES DATE/TIME — 576

# FIG. 21

578

```
┌─────────────────────┐
│   METER READS        │
│   SOFTWARE CONTROL   │
│   BLOCK AND EXTRACTS │
│   SOFTWARE ID        │
└─────────────────────┘
           │
           ▼
580
      ╱─────────╲                    ┌─────────────────┐
     ╱ DECRYPTION ╲      NO          │    DISPLAY       │
    ╱    KEY       ╲────────────────▶│    MESSAGE       │
    ╲  AVAILABLE?  ╱                 │  NEW PROGRAM     │
     ╲           ╱                   │  MUST BE ADDED   │
      ╲─────────╱                    └─────────────────┘
           │ YES
           ▼
582
      ╱─────────╲                    ┌─────────────────┐
     ╱  CURRENT  ╲      NO           │    OBTAIN        │
    ╱ RESOURCE LIMITS╲──────────────▶│ AUTHORIZATION    │
    ╲ SUFFICIENT?   ╱                │ FROM CENTRAL     │
     ╲            ╱                   │  COMPUTER        │
      ╲─────────╱                    └─────────────────┘
           │ YES
           ▼
584
      ╱─────────╲                    ┌─────────────────┐
     ╱  COST OR  ╲      YES          │    DISPLAY       │
    ╱ TIME NEAR   ╲────────────────▶│    WARNING       │
    ╲  MAXIMUM    ╱                  │  ADDITIONAL      │
    ╲   VALUE?   ╱                   │ AUTHORIZATION    │
     ╲─────────╱                     │  REQUIRED        │
           │ No                      └─────────────────┘
```

```
┌─────────────────┐
│   METER READS    │
│   SOFTWARE_      │
│   COST_TABLE     │
│   FROM           │──── 586
│   SOFTWARE       │
│   CONTROL        │
│   BLOCK          │
└─────────────────┘
         │
         ▼
┌─────────────────┐
│    METER         │
│  APPLIES ANY     │
│  OVERRIDES OR    │
│ ADJUSTMENTS      │──── 588
│  FROM ITS        │
│  INTERNAL        │
│  COST_TABLE      │
└─────────────────┘
         │
         ▼
┌─────────────────┐
│  METER AND       │
│   GAME           │
│  COMPUTER        │
│  TRANSFER        │
│  CONTROL TO      │
│  SECURE          │──── 590
│  SOFTWARE        │
│  COMPONENT       │
│ AND INSECURE     │
│  SOFTWARE        │
│  COMPONENT       │
└─────────────────┘
```

# FIG. 22



GAME COMPUTER EXECUTES INSECURE SOFTWARE COMPONENT

COMMUNICATE AT LEAST ONE INPUT PARAMETER TO METER

METER EXECUTES SECURE SOFTWARE COMPONENT AND GENERATES AT LEAST ONE OUTPUT PARAMETER THAT IS COMMUNICATED TO GAME COMPUTER

METER CREATES RECEIPT ENTRY, SIGNS IT WITH SK_METER, AND APPENDS IT TO THE RECEIPT FILE — 592

METER UPDATES TOTAL_COST BASED ON EVENT BEING BILLED — 594

— 596

LIMIT_TIME OR LIMIT_COST EXCEEDED?

No

YES

METER STOPS EXECUTING SECURE SOFTWARE COMPONENT

# FIG. 23

METER CREATES USAGE
REPORT MESSAGE:
TOTAL COST, RECEIPT
FILE (OPTIONAL) — 598

CENTRAL COMPUTER
UPDATES BILLING
INFORMATION FOR
ACCOUNT — 600

CENTRAL COMPUTER
RETURNS USAGE
REPORT
ACKNOWLEDGMENT
MESSAGE — 602

METER DISPLAYS
TOTAL_COST BEING
BILLED — 604 / 612

METER SIGNALS GAME
COMPUTER TO RENAME
THE RECEIPT FILE TO A
BACKUP FILE AND TO
CREATE A NEW BLANK
RECEIPT FILE — 606 / 614

IF COSTS ARE CALCULATED
BY CENTRAL COMPUTER

METER CREATES USAGE
REPORT MESSAGE:
USE_COUNT_TABLE
USE_TIME_TABLE
RECEIPT FILE
(OPTIONAL) — 608

CENTRAL COMPUTER
CALCULATES COSTS
AND RETURNS USAGE
REPORT
ACKNOWLEDGMENT
MESSAGE — 610

# FIG. 24

616 — 
```
┌─────────────────────┐
│   METER GENERATES   │
│  RECEIPT FILE CHECK │
│      MESSAGE        │
└─────────────────────┘
```

618 — 
```
┌─────────────────────┐
│  CENTRAL COMPUTER   │
│  COMPARES RECEIPT   │
│   FILE TO BILLING   │
│   INFORMATION IT    │
│ RECEIVED FROM METER │
└─────────────────────┘
```

620 — 
```
┌─────────────────────┐
│  CENTRAL COMPUTER   │
│ CREATES RECEIPT FILE│
│ VALIDATION MESSAGE  │
└─────────────────────┘
```

622 — 
```
┌─────────────────────┐
│   METER DISPLAYS    │
│  INFORMATION FROM   │
│  CENTRAL COMPUTER   │
│ AND EXPLANATION FOR │
│  ANY DISCREPANCIES  │
└─────────────────────┘
```

# FIG. 25

624 — METER CREATES AUTHENTICATABLE OUTCOME MESSAGE AOM BY ENCRYPTING OUTCOME WITH SK_METER

626 — CENTRAL COMPUTER RECEIVES AND AUTHENTICATES THE AOM BY DECRYPTING THE AOM WITH SK_METER

628 — CENTRAL COMPUTER RETURNS AUTHENTICATED OUTCOME CONFIRMATION MESSAGE AOCM TO METER

630 — METER DISPLAYS CONFIRMATION OR DENIAL OF OUTCOME ACCEPTANCE

SUBSTITUTE SHEET (RULE 26)

# FIG. 26

632 — METER DISPLAYS GAME OUTCOME, ID_METER

634 — PLAYER DIALS CENTRAL COMPUTER, CONNECTS, AND ENTERS GAME OUTCOME, ID_METER IN RESPONSE TO PROMPTS FROM IVRU

636 — CENTRAL COMPUTER GENERATES AND COMMUNICATES RANDOM CHALLENGE STRING TO PLAYER, WHO ENTERS IT INTO METER

638 — METER CALCULATES CRYPTOGRAPHIC HASH OF GAME OUTCOME AND CHALLENGE STRING AND ENCRYPTS IT WITH SK_METER TO FORM AN AUTHENTICATABLE OUTCOME MESSAGE AOM, METER DISPLAYS AOM

PLAYER ENTERS AOM INTO TELEPHONE KEY PAD, FROM WHERE IT IS COMMUNICATED TO CENTRAL COMPUTER — 640

CENTRAL COMPUTER RECEIVES AOM AND AUTHENTICATES AOM BY INDEPENDENTLY CALCULATING HASH AND ENCRYPTION — 642

CENTRAL COMPUTER RETURNS AUTHENTICATED OUTCOME CONFIRMATION MESSAGE AOCM TO PLAYER, PLAYER ENTERS AOCM INTO METER — 644

METER DISPLAYS CONFIRMATION OR DENIAL OF OUTCOME ACCEPTANCE — 646

FIG. 28



FIG. 27

# INTERNATIONAL SEARCH REPORT

| International application No. |
| --- |
| PCT/US96/18834 |

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)  : H04L 9/32; G06F 161:00
US CL  : 380/23, 25; 463/29

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S.  :  380/23, 25; 463/29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| --- | --- | --- |
| X,P | US 5,508,731 A (KOHORN) 16 April 1996, col. 14, lines 6-49; col. 19, lines 13-18; col. 25 lines 46-58; col. 26, lines 51-68. | 1-28 |
| X | US 5,297,205 A (AUDEBERTet al) 22 March 1994, col. 2, lines 18-35, col. 7, lines 5-41; col. 13, lines 20-28 | 1-28 |
| X | US 5,073,931 A (AUDERBERT et al) 17 December 1991, col. 6, lines 26-57, col. 8, lines 52-55; col. 11, lines 19-31. | 1-28. |
| Y | US 5,243,652 (TEARE et al) 07 September 1993, col. col. 2, lines 36-49. | 16 |
| Y | US 5,202,923 A (KURIYAMA) 13 April 1993, col. 6, lines 32-49. | 10 |

| X | Further documents are listed in the continuation of Box C. |  | See patent family annex. |

* Special categories of cited documents:

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| Date of the actual completion of the international search | Date of mailing of the international search report |
| --- | --- |
| 04 MARCH 1997 | 2 5 MAR 1997 |

| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | Authorized officer  *[signature]*<br>GILBERTO BARRON |
| --- | --- |
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Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A,P | US 5,539,822 A (LETT) 23 July 1996, col. 21, lines 5-14. | 28 |

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS
search terms: interactive and game#; and ranking#; game and video; encod?(2a)(outcome or result); authenticat?; authenticat?(w)outcome; authenticat?(w)result

# PCT

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
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(81) Designated States: CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

**Published**
*With international search report.*

---

(54) Title: A 46 Kd HUMAN MILK FAT GLOBULE ANTIGEN

(57) Abstract

A polypeptide has the antibody binding activity of the 46 Kdalton HMFG antigen and/or homology to at least one of the light chains of clotting factors V and VIII and/or contains RGD and/or EGF-like segments. The polypeptide is provided as a recombinant and/or glycosylated and/or fusion protein. An antiboy has high affinity for specificity epitopes of the polypeptide of the invention. Polynucleotide segments encode the polypeptide, recombinant and fusion protein of the invention or fragments thereof, and immunoassay kits comprise the antibodies and/or polypeptides of the invention and other components. In vivo, ex vivo, and in vitro methods of therapy, vaccination and diagnosis utilize the polypeptide, fusion protein anti-sense nucleotides, antibodies and/or polynucleotides of the invention.

## A 46 Kd Human Milk Fat Globule Antigen

5                              BACKGROUND OF THE INVENTION

### Field of the Invention

This invention relates to the field of diagnosis and therapy of cancer and the prevention and treatment of viral infections. More particularly, it relates to a polypeptide having the antibody binding specificity of the 46 Kdalton HMFG antigen, 10  hybrid protein thereof, anti-idiotype antibodies and polynucleotides, anti-sense polynucleotides encoding them, kits, and their application to the in vitro detection, the in vivo and ex vivo of delivery of a therapeutic agent, the detection of the polynucleotides by hybridization with labeled probes, and the vaccination against and treatment of cancer and viral infections.

15  ### Description of the Background

The human milk fat globule (HMFG) has been used extensively as a source of antigenic material for the preparation of both polyclonal and monoclonal antibodies that have found widespread use in the diagnosis of breast cancer, as well as in the study of the breast epithelial cell surface and the processing of its antigenic components.

20        Polyclonal antiserum was originally prepared, that after appropriate absorptions with non-breast tissue was found to identify surface antigens of human mammary epithelial cells (HME-Ags). This antiserum (anti-HME) had a high specificity for normal breast epithelial cells and breast carcinomas. It identified mainly three components of the human milk fat globule which had molecular weights of 150 Kdalton, 70 Kdalton, 25  and 46 Kdalton, respectively.

Monoclonal antibodies were first made against the HMFG in 1980. These antibodies were applied to identify a hitherto unknown component of the breast epithelial cell surface, a large molecular weight mucin-like glycoprotein, that was named non-penetrating glycoprotein (NPGP). This latter component appears to be 30  extremely antigenic in the mouse. The vast majority of monoclonal antibodies prepared against HMFG as well as breast tumors have been found to have specificity against different epitopes of this mucin. Less frequently, monoclonal antibodies have been prepared against the 70 Kdalton and 46 Kdalton components of the HMFG.

The reason for the high immunogenicity of NPGP was elucidated by the 35  characterization of cDNA clones selected from a λgt11 breast cell library using both polyclonal and monoclonal antibodies against the mucin. These cDNA clones consist

1

of large arrays of highly conserved 60 bp tandem repeats. The resulting 20 amino acid repeat contains epitopes for several anti-mucin antibodies. The repeat is apparently unstable at the genomic level. This may account for the observed polymorphism seen at the gene, RNA and protein levels for this high molecular weight mucin. An initial

5  report on cDNA cloning of the mucin product suggested that the core protein had a molecular weight of about 68 Kdalton. However, the mRNA was found to be large enough to code for proteins from about 170 Kdalton to 230 Kdalton. More recently, using milder deglycosylation methods, a core protein was identified having a molecular weight of about 200 Kdalton. Attention has also been devoted to the study and use

10  of the NPGP mucin, largely as a result of its high immunogenicity. Thus, a large number of monoclonal antibodies were prepared against it. However, the smaller components of HMFG also appear to be important molecules on the surface of breast epithelial cells. They have a breast specificity as demonstrated by the anti-HMFG antibodies.

15       The 46 Kdalton and 70 Kdalton HMFG antigens are found in serum of breast cancer patients and thus can be used as markers for breast cancer in serum assays. In addition, the 70 Kdalton component has been found to co-purify with the intact mucin complex and has been reported to be associated with the NPGP mucin complex by means of disulfide bonds, making it a possible linker protein of this surface mucin

20  complex. Polyclonal antibodies against a major component of the HMFG having molecular weight of 155 Kdaltons have been prepared. It was found that antisera bound also to the apical surface of lobules and terminal ducts, but not to the larger ducts of the mammary gland. The latter also did not bind to the apical surface of normal apocrine and eccrine sweat gland coils and ducts, or sebaceous glands in skin.

25  The HMFG-GPI55 did become localized in Paget's disease and breast disease but not in cases of extramammary disease.

        Few monoclonal antibodies, however, have been prepared against the smaller components of the HMFG system, such as the 70 Kdalton and 46 Kdalton HMFG antigens. The breast mucin glycoprotein molecule appears to be highly antigenic

30  because of its internally repeated structure. The components of the mucin glycoprotein was recently determined and a partial sequence for the 70 Kdalton antigen obtained by cDNA cloning. A role for the 70 Kdalton antigen has been suggested as a linker protein for the breast mucin. The 46 Kdalton component of the HMFG system has been found to be present in the serum of breast cancer patients. In addition, with the

35  aid of both monoclonal and polyclonal antibodies against the 46 Kdalton HMFG antigen, circulating immune complexes of the 46 Kdalton HMFG antigen were detected in breast

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cancer patients, and an increase in the circulating 46 Kdalton HMFG antigen was found to be associated with tumor burden.

Accordingly, there is still a need for an improved product and methods suitable for diagnostic and therapeutic applications to human cancer and virus-associated

5   infections.


## SUMMARY OF THE INVENTION

This invention relates to a pure, isolated polypeptide having the antibody binding specificity of the 46 Kdalton HMFG antigen and/or homology to at least a portion of a light chain of clotting factors V and VIII, and/or RGD and/or EGF-like segments, and

10   to a composition comprising the polypeptide and a biologically acceptable carrier, e.g. a pharmaceutically-acceptable carrier. The naked polypeptide may be produced by recombinant cloning and expression in prokaryotes and the glycosylated version in eukaryotes. The polypeptide of the invention is also provided, with a second antigenic polypeptide bound thereto, as a fusion protein and as a composition comprising the

15   hybrid protein and a biologically acceptable carrier, e.g. a pharmaceutically-acceptable carrier. An antibody detecting kit provided comprises, in separate containers, the polypeptide of the invention or a functional fragment thereof, anti-constant region immunoglobulin or protein G or A or fragments thereof, and instructions for its use. Another kit comprises, in separate containers, the fusion protein of the invention

20   comprising a second antigenic polypeptide, or an anti-second polypeptide polyclonal or monoclonal antibody, and anti-constant region immunoglobulin, protein G or A or binding fragments thereof. The polypeptide of this invention or a binding fragment thereof may also be applied to the vaccination of a mammal such as a human in an amount and under conditions effective to raise antibodies which are capable of

25   selectively binding to the 46 Kdalton HMFG antigen, functional fragments, or cells carrying them. Yet another application for the polypeptide of the invention is in the in vitro detection of circulating anti-46 Kdalton HMFG antigen antibody. This can be attained by adding the polypeptide of the invention or a functional fragment thereof to a sample under conditions effective to form an antibody-polypeptide complex, and

30   determining the presence of any complex formed. The polypeptide of this invention or a functional fragment thereof is also useful for the therapeutic treatment of viral infections such as those associated with the HIV and rotavirus, among others. This may be attained by, e.g. feeding a subject the polypeptide of the invention or a functional fragment thereof in an amount and under conditions effective to treat or

35   prevent the viral infection. The polypeptide may be utilized as such or in glycosylated

3
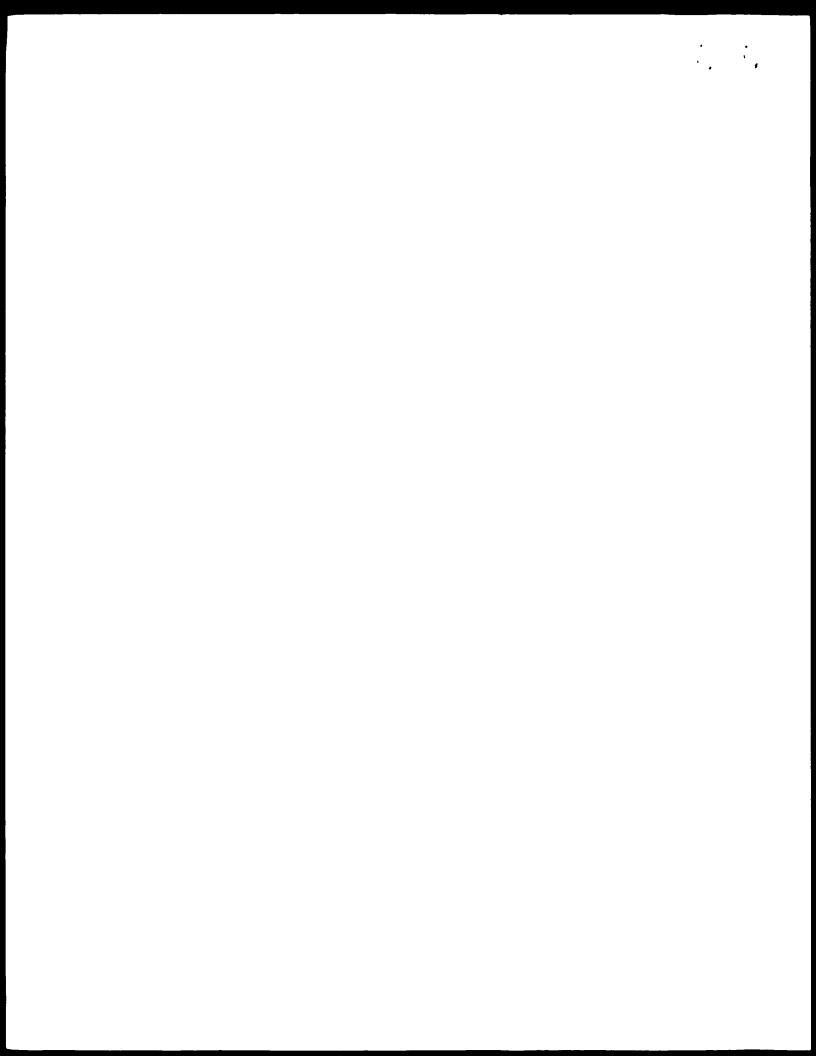
form. Another way of detecting the presence of circulating anti-46 Kdalton HMFG antigen antibody is by contacting a sample with the fusion protein of this invention to form an antibody-fusion protein complex, then adding an anti-second polypeptide antibody to form a double antibody-fusion protein complex, and determining the

5    presence of any double antibody complex formed. The assay may be a solid-phase assay, e.g. where the fusion protein is attached to a solid support.

Still part of this invention are antibodies having high selectivity, affinity and specificity for the 46 Kdalton HMFG antigen, and as anti-idiotype antibodies, and to a composition comprising the antibodies and a biologically acceptable carrier, e.g. a

10   pharmaceutically-acceptable carrier. The antibodies are also provided as an immunoassay kit that comprises, in addition, in separate containers, the monoclonal antibody having high affinity, selectivity and specificity for the 46 Kdalton HMFG antigen or a functional fragment thereof, an anti-constant region immunoglobulin or protein G or A or fragments thereof, and instructions for its use. Also encompassed

15   by this invention is an anti-cancer kit comprising, in separate containers, a monoclonal antibody having specificity for the 46 Kdalton HMFG antigen, and an anti-cancer therapeutic agent selected from the group consisting of immunotoxins and radionucleides, among others. The antibodies selectively binding the 46 Kdalton HMFG antigen are useful for detecting the presence of cancer cells, the polypeptide or

20   fragments thereof in a biological sample such as milk, serum and the like. They may be added to a biological sample of cancerous origin to form an antibody-polypeptide complex, and then determining the presence of any complex formed. The antibodies may also be applied to determining the presence of circulating epithelial cells in a biological sample by adding them to the sample under conditions effective to form an

25   antibody-cell polypeptide complex, and determining the presence of any complex formed. Cells that express the polypeptide of the invention or fragments thereof may be imaged by administering to a subject suspected of being afflicted with cancer or under cancer therapy the anti-46 Kdalton antibody of the invention under conditions effective to deliver it to target body cells expressing the 46 Kdalton HMFG antigen or

30   fragments thereof to form an antibody-cellular antigen complex, then administering to the subject a detectable labeled molecule capable of binding to the antibody at a site other than its binding site for the cellular antigen, and non-invasively detecting the presence of label in the subject's body associated with any complex formed.

This invention also relates to polynucleotides encoding the polypeptide described

35   herein or antibody binding fragments thereof as well as polynucleotides encoding the fusion protein of the invention or antibody binding fragments thereof, DNA segments

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which are complementary to the polynucleotides provided herein, and hybrid polynucleotides, hybrid vectors and host cells transfected with the vectors. The DNA and RNA segments of the invention may be used for the production of the polypeptides and in a method of detecting the presence of a polynucleotide segment encoding the

5    polypeptide described above or fragments thereof  by hybridization under pre-set conditions.  A group of polynucleotides comprising an anti-sense segment to a polynucleotide encoding the polypeptide of the invention or antibody binding fragments thereof of about 15 to 3000 bases.  These polynucleotides are suitable for treating breast cancer by their administration to a patient therapeutic amount.  A therapeutic

10   agent may be delivered in vivo to target cells expressing the 46 Kdalton HMFG antigen or a  functional fragment thereof by administering it to a subject suspected of carrying target cells, such as malignant tumor cells, in a therapeutic amount operatively linked to the anti-46 Kdalton HMFG antigen antibody at a site other than the antigen's binding site under conditions effective for reaching the target cells environment, and allowing

15   the antibody to bind to the cells and the therapeutic agent to act upon the cells. A therapeutic agent may also be delivered ex vivo to target cells expressing the 46 Kdalton HMFG antigen or a functional fragment thereof by, contacting the anti-46 Kdalton HMFG antigen antibody carrying the therapeutic agent with a sample containing the target cells, such as cancer cells under conditions effective to promote

20   the formation of antibody-cell polypeptide complexes,  separating any complexes formed, and returning the sample to the subject.

A more complete appreciation of the invention and many of the intended advantages thereof will be readily perceived as the same becomes better understood by reference to the following detailed description when considered in connection with

25   the accompanying figures.

5

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the expression of BA46-1 specific mRNA in human carcinoma cell lines. Total RNA (20 $\mu$g/lane) was run on a 1.4% agarose gel, blotted, and hybridized to $^{32}$P labelled RNA generated from the BA46-1 cDNA clone. The contents

5 of the samples in the different lanes are as follows: a) A549 (lung); b) BT20 (breast); c) ELLG (breast); d) Raji (lymphoid); e) SKBR3 (breast); f) SKOV3 (ovary); g) MDA-MB-361 (breast); h) MDA-MB-331 (breast) i) HeLa (cervix); j) HS578T (breast); k) HT29 (colon); l) PanCl (pancreas); m) MCF7 (breast). Exposure was 16 hours with an intensifying screen.

10 Figure 2 shows a dendrogram of the aligned C-type domains for various related proteins.

Other objects, advantages and features of the present invention will become apparent to those skilled in the art from the following discussion.

## BEST MODE FOR CARRYING OUT THE INVENTION

15 This invention arose from a desire by the inventors to improve on technology useful for the detection, diagnosis, and treatment of breast cancer of epithelial origin and/or prevent viral infections. Monoclonal antibodies against the 46 Kdalton HMFG antigen have also shown some effectiveness in the radioimmunotherapy of transplanted human breast tumors in experimental animals nude mice. Moreover, an

20 impure preparation of the 46 Kdalton HMFG antigen also been implicated in the inhibition of viruses, such as rotaviruses which are infectious agents causing gastroenteritis, particularly affecting infants, young children and immunologically compromised patients. This work relies on the isolation of cDNA clones that encode partial DNA sequences of the 46 Kdalton apparent molecular weight (app. MW)

25 polypeptide component of the HMFG system and of monoclonal antibodies that bind the 46 Kdalton component of the human milk fat globule (HMFG) system with high affinity and selectivity. The HMFG membrane system, in fact, truly represents a purified portion of the apical surface of the normal breast epithelial cell. The 46 Kdalton app. MW component is a major molecular species of the HMFG membrane and

30 represents a major and important component of the apical surface of the normal breast epithelial cell. Nucleotide and deduced amino acid sequences of a partial cDNA fragment obtained first are shown in Table 1 of Example 7 below. The partial amino acid sequence of the encoded polypeptide is about 217 amino acids long, has a theoretical MW of about 25 Kdaltons and represents the C-terminus of the 46 Kdalton
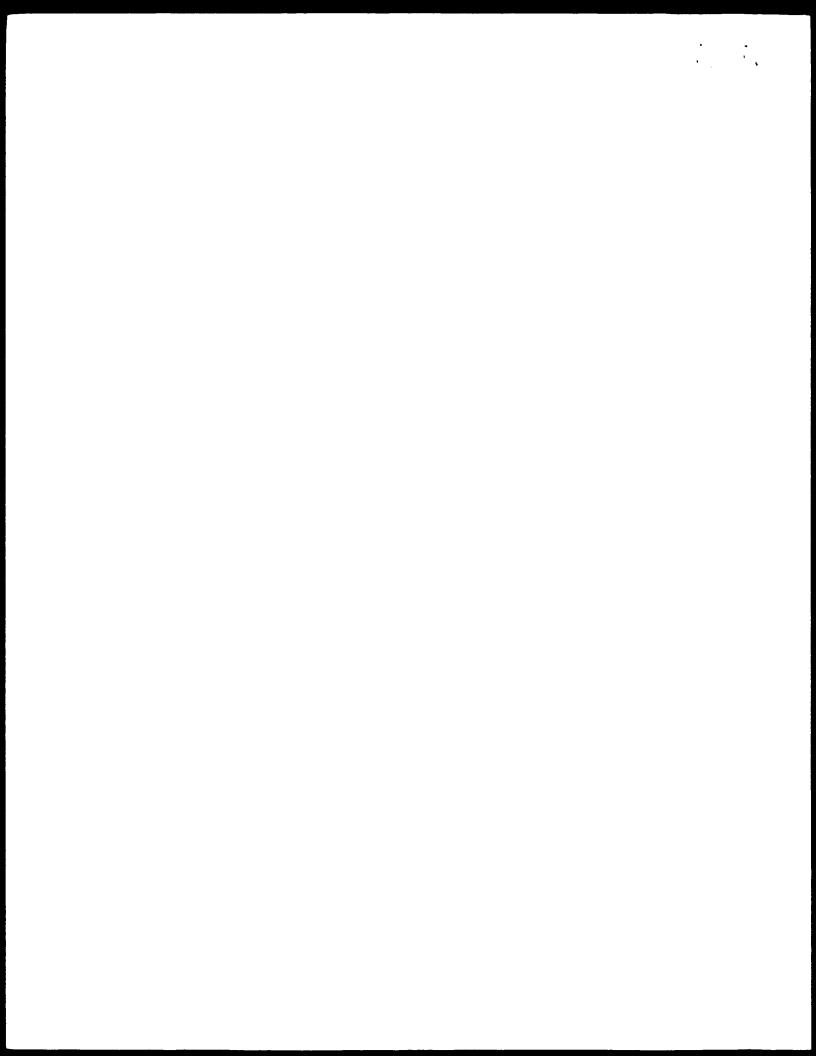
HMFG antigen. This fragment contains four potential sites for N-linked glycosylation and is asparagine and leucine rich. Starting from the C-terminus, the nucleotide sequence extends to the 3' end of the mRNA which contains the AATATA consensus sequence preceding the poly-A segment for cleavage and polyadenylation. A

5   comparison of the C-terminal nucleotide sequence to sequences in the EMBL database using FSTNSCAN (PCGENE) revealed extensive homology with human serum factors V and VIII, and with protein C. The C-terminal deduced protein sequence, however, shares identity only with factors V and VIII but not with protein C since the homology at the nucleotide level is found in an intervening sequence (See, Table 2 below). There

10  is also an about 43% identity to factor V and about 38% identity to factor VIII. The regions of factors V and VIII shown in Table 2 share an about 47% identity with the fragment of the protein shown. The results of the analysis of the deduced amino acid sequence of the C-terminal 46 Kdalton antigen fragment are consistent with it being a glycosylated protein. Its homology to clotting factors V and VIII may be found in the

15  C1C2 region of the light chain of factor VIII. Human antibodies that bind this region of the light chain of factor VIII inhibit the factor by preventing its interaction with phospholipids, and since this region of factor VIII has been implicated in phospholipid binding, it is likely that the homologous region in the C-terminus of the 46 Kdalton HMFG polypeptide serves a similar role. The appearance of a shared domain in

20  otherwise different proteins may be due to exon shuffling. The C-terminus of the 46 Kdalton HMFG antigen may serve as a novel "anchor" sequence for the 46 Kdalton HMFG protein or it may be involved in the binding of mucin and/or cell membranes to the phospholipids found on the surface of growing milk fat droplets. Alternatively, the homologous sequence may be involved in the assembly of the mucin complex at the

25  plasma membrane surface.

The single stranded RNA probe provided herein is complementary to the ORF found in the cDNA insert, that is in frame with the ß-galactosidase DNA sequence in the λgt11 vector. This ORF, therefore, represents the sense strand of the C-terminal portion of the gene since only the complementary strand probe binds to a specific 2.2

30  kilobase mRNA of epithelial cell lines. The cDNA sequence encoding the C-terminus of the 46 Kdalton HMFG glycoprotein was reported in the original text of this patent, and its deduced amino acid sequence showed to have extensive sequence similarity with the C1C2 domain of human coagulation factors V and VIII (43% and 38% respectively). Upon further searching, other proteins were found that have sequences

35  similar to the C1C2 domain of factors V and VIII. These include a neuronal recognition molecule (A5 antigen) of Xenopus (Takagi et al, Neuron 7:295 (1991)), discoidin I of
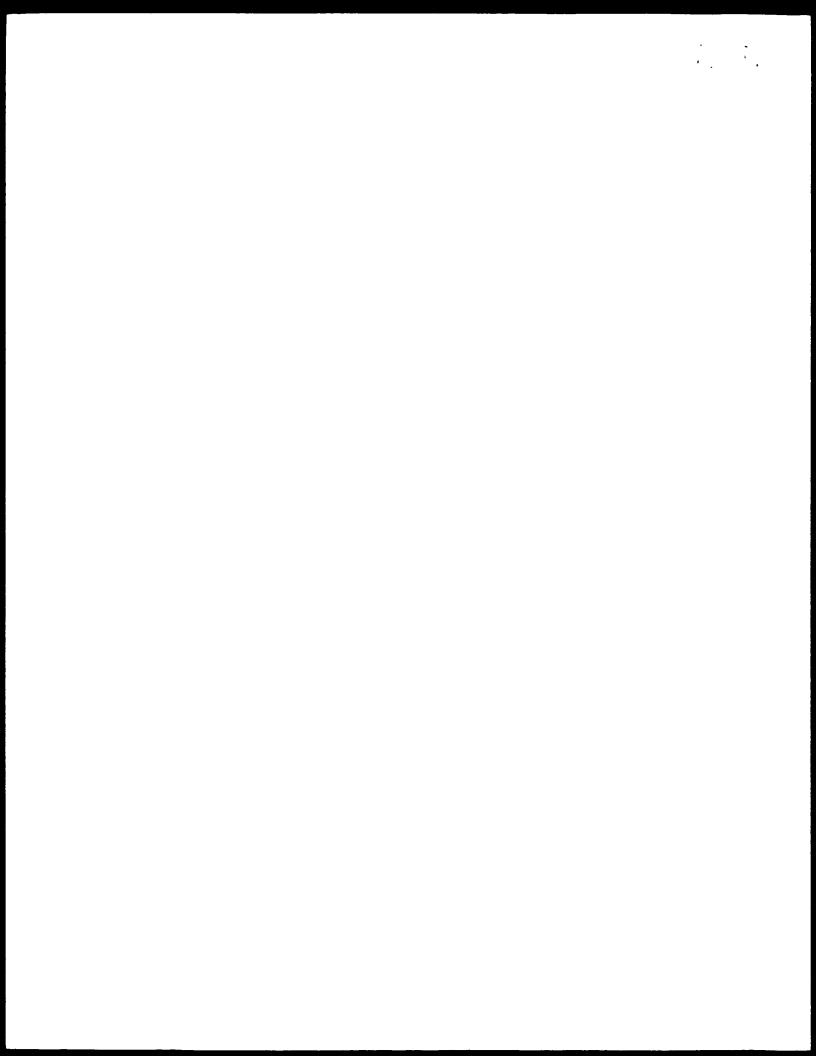
Dictyostelium discoideum (Poole et al, PNAS (USA) 90:5677 (1993)), a receptor
tyrosine kinase with an extracellular discoidin I-like domain (Johnson et al, PNAS (USA)
90:5677 (1993)), a 63/55 Kdalton glycoprotein of the mouse milk fat globule (Stubbs
et al, PNAS (USA) 87:8417 (1990)), and components 15/16 and GP55 of bovine and

5    guinea-pig milk fat globule (Mather et al, Biochem. Mol. Biol. Int. 29:545 (1993)),
respectively. Homologous portion of their sequences are shown in Table 6 below.
When the complete gene was sequenced, it was found that the largest open reading
frame of the BA46 cDNA clone encodes a protein of about 387 amino acids with an
estimated molecular weight of about 43,123 Kdaltons. The actual correspondence of

10   the cDNA cloned to the 46 Kdalton HMFG glycoprotein antigen isolated from the HMFG
is shown by correlating the levels of mRNA with that of the expressed the 46 Kdalton
HMFG antigen in different breast cell lines, and the binding of the monoclonal
antibodies used in the cDNA screening to the pEX/LB21 fusion protein expressed in E.
coli. In addition, five defined and distinct epitopes in the C-terminal end of the protein

15   were determined by epitope mapping for two monoclonal antibodies of the cocktail
used in the original screening of the cDNA library (Mc8 = DPRTG, and Mc16 = SSKIF)
(See, Table 4 below). The two other monoclonal antibodies (Mc3, Mc15) neither bound
to the fusion protein nor to any of the peptide hexamers used in the epitope mapping
of the C-terminal region (amino acids 330 - 382) of the 46 Kdalton polypeptide.

20   However, the two monoclonal antibodies, Mc3 and Mc15, bound to the full length
recombinant polypeptide produced by expression in bacteria of the complete cloned
cDNA sequence encompassing the entire ORF but not the signal peptide.

The amino acid sequence of the polypeptide deduced with the help of the
PC/GENE DNA and the protein analysis programs (IntelliGenetics, Inc.) revealed the

25   existence of homologies or sequence similarities with several functional domains. At
the N-terminal end of the polypeptide, there is a hydrophobic region positioned after
the Met start codon which most likely corresponds to a signal peptide. Cleavage most
likely occurs between $Val_{21}$ and $Ala_{22}$, leaving a cleaved peptide of 21 amino acids plus
the methionine. This cleavage results in a processed polypeptide of about 40,862

30   Kdaltons. Amino acids 46 to 48, RGD, represent a known cell adhesion sequence, and
following this is an EGF-like domain of approximately 12 amino acids encompassing
amino acids 55 to 66. The C-terminal end of the polypeptide starting at amino acid 69,
comprises a domain with homology to the C1C2 region of human coagulation factors
V and VIII, a portion of which is shown in Table 1 below. This sequence contains four

35   potential N-linked glycosylation sites, all present in the C1C2-like domain, numerous
potential O-linked glycosylation sites, disulfide linkages, and phosphorylation sites (e.g.

protein kinase C and casein kinase II sites). The greatest homology, however, is seen with the 66/55 Kdalton antigen MFGE8 isolated from the mouse milk fat globule (Stubbs et al., supra). These results permit the grouping of the 46 Kdalton HMFG polypeptide with growth factors and other molecules associated with cell adhesion

5    interactions, e.g. associated with breast epithelial cells, that provide a possible autocrine/paracrine function. The 46 Kdalton HMFG antigen thus is likely a selectin-like molecule, which has the general structure of an N-terminal adhesion domain (lectin domain) followed by an EGF-like domain, a variable number of complement regulatory elements, a membrane-association domain (a single transmembrane sequence), and a

10   short cytoplasmic tail. Although the 46-Kdalton antigen appears to lack a trans-membrane domain, the C-type domain is very likely the means by which the 46 Kdalton HMFG antigen associates with the cell membrane by interaction with phospholipids. The possible cell interaction properties may be mediated via the cell adhesion sequence RGD since breast cells are known to possess integrins that have receptors for this

15   sequence. The autocrine/paracrine properties may be mediated by the EGF-like sequence. The 46 Kdalton HMFG polypeptide is abundantly present in the HMFG and the expression of its mouse homologue is increased during lactation. Thus, the expression of the human 46 Kdalton HMFG and its mouse homologue are associated with differentiation in the breast. The production of the molecules is highly increased

20   during lactation. Thus, except for periods of lactation, only cancer cells will express high amount of the 46 Kdalton HMFG antigen. Normal resting breast cells do not stain with anti-46 Kdalton HMFG antigen antibodies, showing the antigen to be substantially absent under these conditions.

Although the antibodies used to select the cDNA were specific to the Kdalton

25   HMFG antigen, and happened to bind to breast carcinomas, the expression of the 2.2 kb mRNA that encodes the 46 Kdalton protein occurs in other cancer cell lines. The broad specificity found for cancers from tissues of different origins is attributable to a deregulation of this gene in neoplastic tumors such as carcinomas but not in normal tissue. Although the 46 Kdalton app. MW HMFG antigen may also be expressed by

30   normal epithelial tissue cells, although at a lower level, it is processed in a way that blocks the epitopes that are exposed in the breast cell version of the polypeptide by, for example, producing alterations in its glycosylation. The HMFG mucin is also expressed in non-breast cancer cells such as non-breast carcinoma cells, but its altered processing in the pancreas, for example, leads to the exposure of different antigenic

35   sites than in the breast.

The fusion protein is useful for assaying the presence of the 46 Kdalton HMFG

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antigen or fragments thereof in sera obtained from cancer patients, such as patients suffering from breast carcinomas and also in milk of nursing mothers, among others. This fusion protein is also useful as an immunogen for generating second generation monoclonal and polyclonal antibodies of increased affinity for the antigen. These
5  antibodies may be used, among other applications, to further study the tissue distribution of this antigen and its involvement in the synthesis of its messenger RNA in improved immunoassays, and in the therapy of cancers of epithelial origin, both in vivo and ex vivo.

Many monoclonal antibodies raised against the C-terminus or the complete 46
10  Kdalton HMFG antigen serve to detect the respective epitopes present on this molecule by radioimmunobinding on HMFG membranes, whole milk, milk fractions, and on cancerous membrane material, such as those obtained from breast cancer patients. These monoclonal antibodies do not stain either normal breast tissue nor any other normal tissue when tested by immunohistology. Since some breast carcinomas have
15  very high levels of mRNA encoding the 46 Kdalton HMFG antigenic component, it is possible that second generation antibodies, both monoclonal and polyclonal made against the fusion protein have different, and possibly improved, specificity for detecting the 46 Kdalton HMFG antigenic component by immunohistopathology.

Northern blots using the cDNA clone in the present work showed that the
20  HMFG 46 Kdalton mRNA is present in most breast carcinoma cell lines tested, and in several non-breast carcinoma cell lines, and was still present but at lower levels in one lymphoid cell line (Raji). However, the expression levels of the 2.2 Kilobase RNA encoding the 46 Kdalton HMFG antigen detected vary considerably even amongst carcinoma cell lines. Carcinoma cell lines such as those from lung cells (A549), ovary
25  cells (SKOV3) and two breast cell lines (Ell-G and HS578T) accumulated much more of the RNA transcript than other carcinoma cell lines. In other cases, such as in that of Her 2/neu, and the EGF-like receptor in breast and other carcinomas, the overexpression of certain genes has been correlated with prognosis of the disease. The overexpression of the 46 Kdalton HMFG antigen in neoplastic cells such as
30  carcinoma cells in thus correlatable with the development of cancer disease. Clearly, the 46 Kdalton HMFG antigen was shown herein to evidence epithelial specificity. However, certain epitopes of the 46 Kdalton HMFG antigen may have broader specificity for types of breast cells other than epithelial cells. The 46 Kdalton HMFG polypeptide is expressed significantly in malignant cells such as carcinoma cells due to
35  the deregulation of expression associated with malignancy. The 46 Kdalton HMFG antigen mRNA is highly expressed in cancer cells such as carcinoma cells of breast and

other origins.  This is in contrast to its absence, in a form that is immunologically recognizable, from the corresponding normal cells.

Having cloned a portion of the cDNA of this molecule permitted the further deduction of the sequence of the encoded polypeptide C-terminus.  It also permitted

5   the pursuit of further clones leading to the synthesis of recombinant DNA segments, polypeptides and fusion proteins containing the partial, and ultimately the complete 46 Kdalton amino acid sequence and fragments thereof as well as the preparation of a new generation of monoclonal antibodies against specific epitopes of this polypeptide. The hybrid DNA and fusion protein of the invention permitted the preparation of

10   polyclonal and monoclonal antibodies against the fusion protein of even greater specificity and/or affinity for any particular type of tissue, such as neoplastic cells of a specific organ or cancer type origin.  The various cDNA clones obtained after the sequencing of the C-terminus allowed the deduction of the amino acid sequence of the 46 Kdalton app. MW HMFG antigen component of the HMFG system but the original

15   work led solely to a segment of slightly over 800 bases (217 amino acids) long before the appearance of an EcoR1 restriction enzyme site sequence, unbeknownst to the inventors, precluded its extension.  The remaining portion, representing its N-terminus, would only be arrived at after a lengthy procedural path and trying numerous different methods.

20   The cDNA clones encoding the C-terminal 46 Kdalton HMFG antigen fragment were isolated by screening breast cell λgt11 cDNA libraries using antibodies against the 46 Kdalton MW HMFG antigen.  These libraries were made, as are most other cDNA libraries,  by isolating mRNA from the breast cells, preparing cDNA using poly-dT primers or random primers and a reverse transcriptase enzyme, cutting with the

25   restriction enzyme EcoRI, and cloning into the λgt11 expression vector at the EcoRI restriction site.  The hybrid λgt11 phages were then used to infect a susceptible bacterial strain, and when plaques developed on  the bacterial lawn spread on petri dishes, the plaques were blotted onto a nitrocellulose membrane, the membranes incubated with anti-46 Kdalton MW HMFG antigen antibodies and the plaques that bound the antibody were visualized by exposure to a photographic plate using a

30   bound the antibody were visualized by exposure to a photographic plate using a radioactively labeled second antibody against the first antibody.  Positive plaques were then picked and their cDNA inserts isolated and sequenced.   Although proven successful in obtaining cDNA fragments encoding the C-terminus of the 46 Kdalton MW HMFG antigen, even after many attempts this method did not facilitate the

35   extension of the DNA sequence in the 5' direction beyond the specific point shown in Table 1 below (the EcoR1 site).  As it was learned much later, after a long road
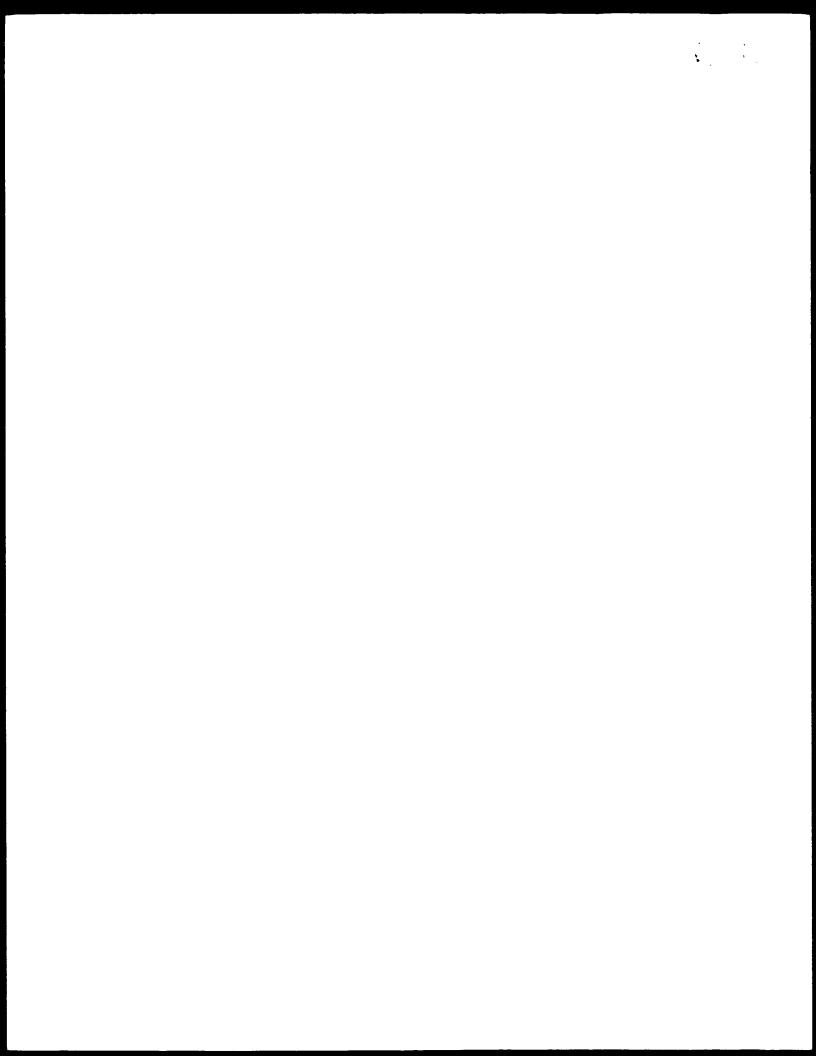
plagued by unsuccessful cul-de-sacs, the major reason for the difficulties encountered in obtaining a full length DNA piece was the presence of an EcoRI restriction site at the specific site of the cDNA encoding the 46 Kdalton MW HMFG antigen represented by the 5' end of the C-terminal fragment isolated initially.  The difficulties encountered

5   which precluded extending the DNA synthesis beyond this EcoRI site are inherent to the manner in which such cDNA libraries are made, and where the DNA fragments are cut and inserted into the λgt11 vector:  at an EcoRI site.  Thus, the 46 Kdalton MW HMFG antigen cDNA fragments in this library encompassed sequences towards the 5' direction to this EcoRI site that  were not obtaineable  with available antibodies

10  recognizing mostly protein epitopes encoded by regions located 3'  to this EcoRI site. The antibodies utilized herein are the sole antibodies ever made by anyone against the 46 Kdalton HMFG antigen.  Therefore, very few, if any, of the 46 Kdalton MW HMFG antigen cDNA clones could be extended beyond this EcoRI site.  In addition, another consequence of the manner in which cDNA fragments are cloned into a phage is a low

15  probability (1 in 6) or a 1:6 proportion of cDNAs cloned in the right direction.  When antibodies are used for screening a cDNA library, their effectiveness is reduced because only 1 out of 6 cDNA inserts in the library will be found to be in the right orientation and proper reading frame to code for the correct protein sequence.  Therefore, the abundance of inserts containing sequences 5' to the EcoRI site in the 46 Kdalton MW

20  HMFG antigen cDNA in these cDNA libraries was much too low to allow their detection and/or isolation.

In addition to the above, another method of screening the hybrid phage library was also tried.  In this method, the libraries were screened with radiolabeled 46 Kdalton MW HMFG antigen cDNAs which, regardless of their orientation or reading

25  frame, would bind to all inserts, but even here the desired sequences were present in amounts too low to be detected and consequently did not lead  to obtaining DNA segments extending beyond this site.  Still another method was utilized, the rapid amplification of cDNA ends (RACE) method to attempt to overcome the stumbling block encountered (Frohman, M.A. et al., PNAS (USA) 85:8998-9002, (1988)).  The

30  RACE protocol generates cDNA fragments by PCR amplification of regions located between a single point in the transcript and either the 3' or the 5' end of the molecule. This is attained with the use of primers specially tailored to these two end regions.  As the RACE method was applied herein, a short stretch of the target cDNA segment had to be known.  Primers oriented in the 3' and 5' directions  were designed, which

35  provided specificity to the amplification step starting from this region.  The extension of the transcribed cDNA fragment starting from the ends of the mRNA was

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accomplished with primers that annealed to the natural 3' end or to an added synthetic

5' end polyA tail. The isolation of the 5' end was attained by means of reverse

transcription with a gene-specific primer. The polyA homopolymer was then appended

to the 5' end of the fragment with the help of terminal transferases. This added a

5   polyA tail to the single stranded cDNA reaction product. The final amplification was

accomplished using a hybrid primer, [oligo-dT of 17 residues linked to a unique 17 base

oligonucleotide ("adaptor") primer], and a second gene-specific primer upstream of the

first one. The amplified sequence was then cloned and sequenced. Although

successful for obtaining some clones of the 3' end, the RACE protocol, even though

10  repeated numerous times, proved unsuccessful to complete the sequence of the cDNA

encoding the 5' end of the 46 Kdalton MW HMFG antigen. In this case, the lack of

success stemmed from the unexpected occurrence of a secondary structure in the 5'

end of the 46 Kdalton MW HMFG antigen mRNA and an inadequate polyA tailing.

       Another method involving the use of PCR technology utilized the direct

15  amplification of cloned cDNAs encoding the 5' end of the 46 Kdalton MW HMFG

antigen from a breast cell λgt11 cDNA library. Utilized as primers in this case were a

downstream primer close to the 5' end of the cDNA encoding the known partial 46

Kdalton MW HMFG antigen and an upstream primer in the λgt11 phage sequence.

These two primers did help amplify inserts containing stretches of the unknown 5'

20  sequences. In this manner, ten amplified cDNAs were isolated, cloned and sequenced.

Disappointingly, however, this proved to be another blind alley since all these clones

had significantly different sequences. Some of these cDNAs appeared to extend the

DNA fragment encoding the 46 Kdalton MW HMFG antigen beyond the impervious

EcoRI restriction site, thus confirming its existence which had only been presumed up

25  to this point from the earlier attempts with antibody screening of the λgt11 cDNA

library, but then all the DNA sequences thus obtained diverged. These spurious results

were probably due to mispriming and amplification of other DNA sequences in the

process.

       An improvement on the RACE method was then implemented that, instead of

30  using polyA tailing, used an AmpliFINDER anchor attached by ligation to the 5' end of

a single stranded cDNA synthesized by reverse transcription of breast cell mRNA.

Another improvement added to the protocol was the use of a heat-stable reverse

transcriptase that is active at 52°C (conventional reverse transcriptases require 42°C).

The higher temperature was used to overcome a previous problem by reducing any

35  secondary structure that might prevent the transcription of the mRNA by the reverse

transcriptase enzyme. The AmpliFINDER anchor was attached to the 5' end and used

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as a site for priming the PCR amplification of sequences between the 5' end and the site of the second primer in the known sequence of the cDNA encoding the 46 Kdalton MW HMFG antigen. The complete DNA sequence of the 46 Kdalton HMFG MW antigen was obtained with this AmpliFINDER RACE protocol and matched with the correct

5   sequence out of the ten sequences obtained from the cDNA library by the PCR amplification method utilized earlier. Thus, the authentic DNA sequence of the unknown portion of the ORF encoding the 46 Kdalton HMFG antigen without the 5' non-coding region was finally obtained.

This clearly illustrates the difficulties encountered and the unexpected and

10  unobvious path travelled to obtain the complete sequence of the cDNA encoding the 46 Kdalton MW HMFG antigen, and the deduced amino acid sequence of the product.

The cDNA clones obtained also allowed the preparation of a new generation of monoclonal antibodies that have sufficient specificity for application to cancer immunotherapy, sufficient staining ability for doing immunohistopathology, greater

15  ability for prognosis, diagnosis, imaging and therapy, and that can better identify the 46 Kdalton HMFG peptide and fragments thereof in the sera of cancer patients and milk of pregnant females and viral-infected patients, among others. The latter property makes these antibodies useful in the diagnosis of cancer and viral infection, and for the screening and early detection of the disease in humans.

20  This invention thus provides a polypeptide having the antibody binding selectivity and specificity of the 46 Kdalton MW HMFG antigen and/or homology to at least one of the light chains of clotting factors V and VIII and/or comprising a RGD and/or EGF-like segment. In one preferred embodiment, the polypeptide has the biological activity of the 46 Kdalton MW HMFG antigen, and more preferably the

25  polypeptide comprises the 46 Kdalton MW HMFG antigen itself or an antibody binding fragment thereof. The polypeptide or fragments thereof may be prepared by recombinant methods, which permit the arbitrary determination of its length and modification to be introduced at the DNA level. The polypeptide of the invention may be about 5 to 1,500 amino acids long, 90 to 500 amino acids long, more preferably

30  about 110 to 280 amino acids long, and still more preferably about 200 to 250 amino acids long. In another preferred embodiment, the polypeptide has the amino acid sequence shown in Table 4 (SEQ. ID No: 6) or that shown in Table 2 (SEQ. ID No: 3) or antibody binding fragments thereof, preferably about 5 to 100 amino acids long, and more preferably about 15 to 50 amino acids long. Particularly preferred are polypeptide

35  fragments which correspond to the specific epitopes which are recognized by the anti-46 Kdalton MW HMFG antigen antibodies prepared in accordance with this invention

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and those containing the RGD and EGF-like segments. The non-glycosylated and glycosylated polypeptide of the invention may be synthetically prepared by methods known in the art such as chemical synthesis and the like. In addition, it may be produced as a non-glycosylated product in bacteria or in glycosylated form in plants or

5   eukaryotic cells or hosts by recloning in appropriate expression vectors and transfection of receptive hosts.
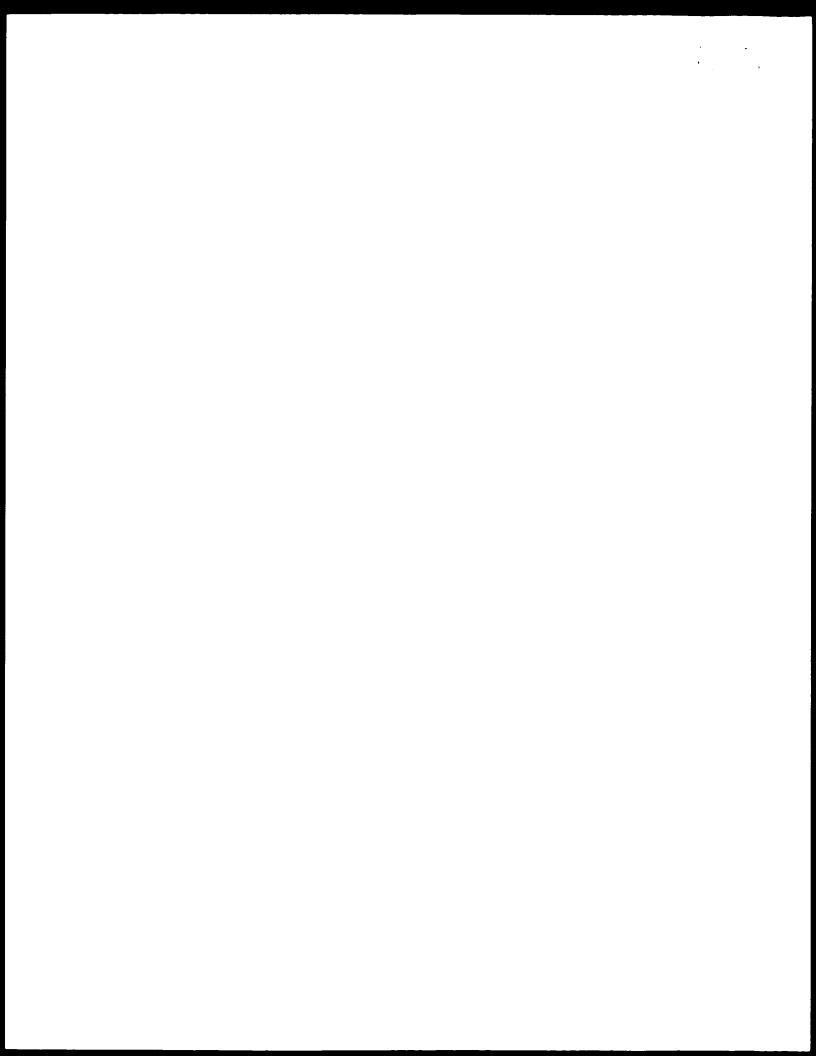
The polypeptide of the invention also has anti-viral properties. Upon fractionation of the human milk fat globules, human milk globule membrane which is the globule's macromolecular component, and its acidic protein fraction retain the anti-

10   viral activity. When the defatted milk fat globule fraction is separated into different fractions, the anti-viral activity of human milk remains mostly with the mucin complex. However, when the mucin complex is separated into its components the highest anti-viral activity is found with 46 Kdalton app. MW HMFG antigen. The 46 Kdalton app. MW HMFG antigen preferentially binds, e.g. simian and human rotaviruses when

15   compared to the 70 Kdalton app. MW HMFG antigen and the 46 Kdalton MW HMFG antigen depleted milk mucin. The human milk fat globules, the macromolecular fraction and the milk mucin complex, which among other fractions contains the 46 Kdalton app. MW HMFG antigen, and the 46 Kdalton app. MW HMFG antigen were all found to inhibit viral infection, e.g., by rotavirus of human and simian origin, of cultured

20   mammalian cells. The mucin complex was shown to inhibit viral infection with a 3000 fold greater specific activity than whole milk. These results are unexpected based on previous ambiguous reports relating to the effect of human milk on rotavirus, and the reported inhibitory effects of other milk components on this virus. The human 46 Kdalton app. MW HMFG antigen was also shown to bind to cells and cell extracts that

25   are infected with human viruses such as rotavirus. Human strains of the virus, such as RRV, Wa, DS-1, P and ST-3, bind to the 46 Kdalton app. MW HMFG antigen in essentially equivalent amounts. Moreover, when sialic acid was removed from the 46 Kdalton app. MW HMFG antigen, its binding to virally infected cells was substantially reduced. This reduction in binding of the 46 Kdalton app. MW HMFG antigen to virus

30   infected cells was found to be in the range of 30 to 60%. Thus, sialic acid may be required for the 46 Kdalton app. MW HMFG antigen to retain its binding activity as well as its anti-viral activity. Moreover, it is also possible that the anti-viral activity of milk mucins from other sources lacking sialic acid may be enhanced by sialylation. The polypeptide of this invention was also shown to inhibit in vitro viral infection of cells

35   as well as viral gastroenteritis induced by viruses, e.g., rotaviruses, in an animal model. For instance, the administration of a murine rotavirus (EDIM) to suckling mice, caused

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a 100% incidence of diarrhea in the mice. However, the simultaneous administration of the virus and the human milk macromolecular or acidic glycoprotein fraction (containing the 46 Kdalton HMFG antigen) to the suckling mice, reduced the diarrhea symptoms by 90%. In contradistinction, when a bovine milk-based formula or a

5  control medium were administered, the rotavirus activity and the diarrheal symptoms remained undiminished. The various components of the human milk fat globule may be purified as described in the art. The polypeptide of this invention may be easily prepared for clinical use either by purification, by recombinant technology or peptide synthesis. The polypeptide may be purified from biological sources as follows. Human

10 breast milk may be readily fractionated by published methods into a macromolecular component comprising the fat globule membrane. This component is distinct from oligosaccharides, lipids, immunoglobulins and other small proteins contained in milk. Likewise, whole human milk, the macromolecular fraction, and the fat globules may be defatted to produce fat globule membranes. The macromolecular fraction containing

15 the milk mucin complex may be obtained by lipid extraction of fatty milk as described by Newburg, D.S., et al. (Newburg, D.S., et al, Pediatric Res. 31:22-28(1992)). The acidic glycoprotein fraction of milk may be obtained by isoelectric focusing as described by Yolken, R.M., et al. (Yolken, R.M., et al, J. Clin. Investigation 90: (1992)). Both these fractions have anti-viral activities that are, respectively, 3 and 38

20 times greater than whole milk. The milk mucin complex may be affinity-purified in accordance with published procedures (Ceriani et al., P.N.A.S.(USA) 74: 582-589 (1977)). Natural skim milk may be prepared by centrifuging unfrozen fresh milk, and removing the cream fraction that contains intact milk fat globules. When fresh milk is frozen and thawed, especially several times, sonicated, allowed to stand for a period

25 of time, or exposed to temperature, the fat globules are generally disrupted. When the fat layer is then separated from the remainder or "processed skim milk", it contains mainly the lipid fraction of the cream (butter consisting of mainly triglycerides), while the milk fat globule membranes, the 70 Kdalton app. MW and the 46 Kdalton app. MW HMFG antigens are now mainly in the "processed skim milk". However, the amount

30 is greatly increased in the "processed skim milk", the amount increasing with more vigorously freezing and thawing and/or sonication. Both the natural and the processed skim milk have anti-viral activity, with the latter evidencing higher activity. Curds and whey may be prepared as is known in the art, and will contain a certain proportion of the described components that have anti-viral activity. The milk mucin complex, in

35 turn, may be further purified from the membranes using monoclonal antibodies as described herein, and the 46 Kdalton app. MW HMFG antigen may be separated from
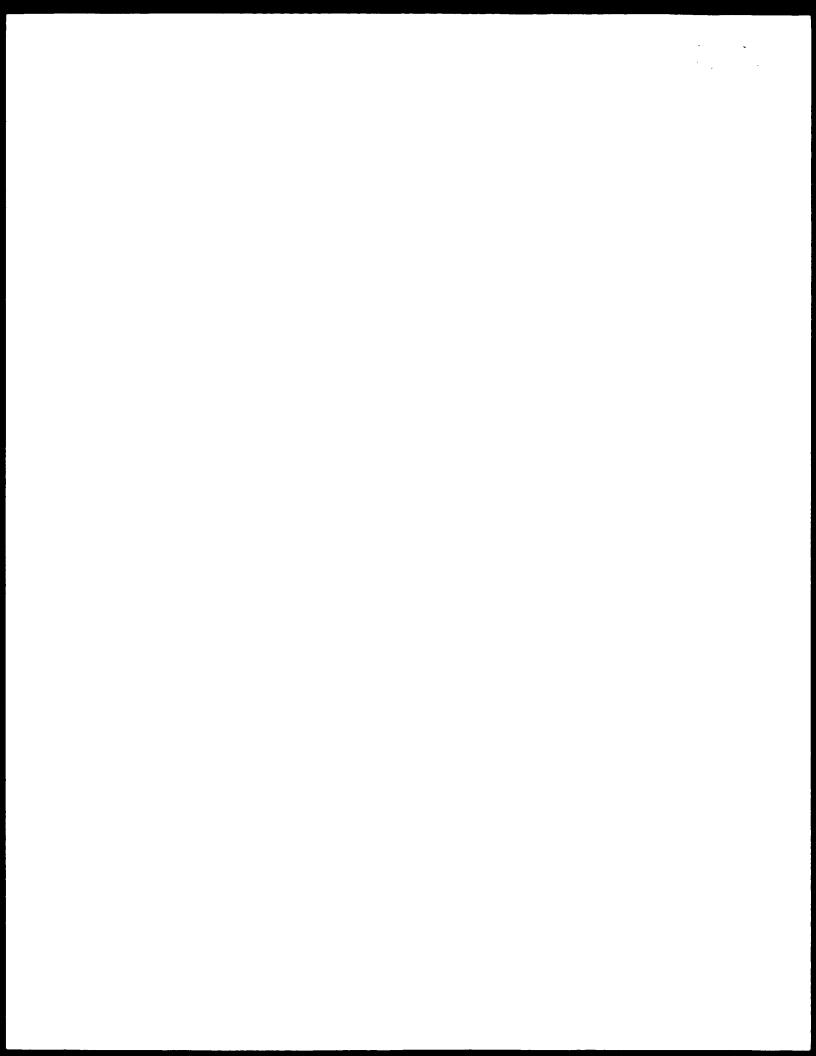
the milk mucin complex or prepared by recombinant technology as described herein or simply by expression in a host, either eukaryotic or prokaryotic, transfected with a hybrid vector carrying the polynucleotide segment of this invention or a fragment thereof. The natural components are separable by traditional chromatographic and/or

5    electrophoretic methods. The presence and identities of the components of the human milk mucin complex are readily determined using available, specific monoclonal antibodies. The gene encoding the 46 Kdalton app. MW HMFG antigen being provided herein, the gene product and variations thereof may be prepared by recombinant technology and expressed in recombinant microorganisms, plant and mammalian hosts

10   as described by Larocca et al. (Larocca et al., Cancer Res. 51:4994 (1991); Larocca et al. Hybridoma 11:191 (1992); Larocca, et al., "Molecular Cloning and Expression of Breast Mucin Associated Antigens"", in Breast Epithelial Antigens, p. 36, Plenum Press, Ceriani, R.L., ed, NY, NY (1991); and others). The amino acid sequence of the 46 Kdalton app. MW HMFG antigen is unrelated to any known immunoglobulin but was

15   found to have significant homology to human epithelial cell proteins, the C1C2 domains of the human clotting factors V and VIII, the cell adhesion sequence RGD and an EGF-like sequence, a mouse milk fat globule 67 Kdalton app. MW protein MFG-E8, discoidin of amoebae, and the A5 antigen of xenopus brain, among others. Polypeptides having the viral binding characteristics of the 46 Kdalton app. MW HMFG antigen or fragments

20   thereof may be prepared synthetically, by expression of genetically engineered vectors in trasfected hosts and/or by adding a stop codon at a desired place in the DNA encoding the protein, by methods known in the art, or by purification from human milk of the 46 Kdalton app. MW HMFG antigen and subsequent partial hydrolysis. The synthetic polypeptide having the described characteristics may be prepared in different

25   lengths by alteration of the DNA sequence encoding it and adding a stop codon where desired, as is known in the art, and expression of the thus altered gene or fragments thereof. The cDNA encoding the 46 Kdalton app. MW HMFG antigen has been cloned and fully sequenced as disclosed herein.

The novel anti-viral agent of this invention is suitable for use in most instances

30   of viral infections, and particularly in cases where other therapies are either ineffective or clinically contraindicated. The agent of this invention exhibits additional advantages for the treatment of infants and children since, as already indicated, its components are normal constituents of human milk and the human diet. The present agent is thus unlikely to elicit toxic, immunological or allergic reactions in treated subjects. Because

35   these agents are innocuous to the human body, the invention may be used without intervention of skilled medical personnel, for example, by adding it to foodstuffs, and

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the like, that are normally sold over-the-counter in convenience stores or as food supplements available in grocery stores. This is a particular advantage for treating travellers or populations in underdeveloped countries where medical services are in short supply. The agent of this invention may be administered in combination with

5   other treatments, such as immune therapy, particularly treatments that act by independent mechanisms, to thereby provide a multi-pronged attack on the virus. Other anti-viral treatments may be combined with the present agent to provide a treatment compatible with other clinical needs of a patient, as well. For example, other milk components, such as oligosaccharides, $a$-interferon and trypsin inhibitors, known to

10  have anti-microbial and anti-viral activity, may be combined with the present agent. The inventors have found that components of human milk other than those encompassed by the invention failed to inhibit rotavirus infection in cell cultures. These agents, prepared by methods described in the art, include lipids, gangliosides, polar neutral glycolipids, non-polar glycolipids, triglycerides and fatty acids and neutral, acidic

15  and total oligosaccharides. The agent of the invention may be used alone, with a carrier or as an additive to a foodstuff, or in other compositions suitable for human consumption. Thus, an anti-diarrheic product may comprise a foodstuff, and an anti-viral effective amount of the polypeptide of the invention, either alone or in combination with other anti-viral agents and/or an agent selected from the group

20  consisting of defatted human milk fat globules, skim milk, the human milk macromolecular fraction, curd, whey, the human milk mucin-70 Kdalton app. MW glycoprotein-46 Kdalton app. MW HMFG antigen complex, and mixtures thereof. Each additional agent may be used alone or combined with one or more of the agents provided herein, or further combined with a foodstuff or food supplement for self-

25  administration. This composition may also be provided with other components including, but not restricted to, vitamin supplements, mineral additives, other nutritional additives, buffers, salts, flavoring compounds, diluents, thickeners, emulsifiers, preservatives, and anti-oxidants, such as would be familiar to a person skilled in the art, as would the amounts they are added in to the composition. The anti-diarrheic

30  composition or product may also comprise a binder such as gum tragacanth, acacia, corn starch or gelatin, excipients such as dicalcium phosphate, anti-clumping agents such as corn starch, potato starch, alginic acid and the like, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose or saccharin, flavoring agents such as peppermint, orange, wintergreen or cherry flavoring as well

35  as other known artificial and natural flavoring compounds. Sustained-release preparations and formulations are also within the confines of this invention, and may

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contain further ingredients as is know in the art. A coated composition, or otherwise modified forms of the preparation are also contemplated herein such as coatings of shellac, gelatin, sugar and the like.

Any material added to this product should be pharmaceutically-acceptable and
5    substantially non-toxic in the amounts employed. Other excipients may be added to the formulation such as those utilized for the production of ingestible tablets, troches, capsules, elixirs, suspensions, syrups and wafers, among others and the product may then be provided in these forms. In one preferred embodiment, the polypeptide, whether glycosylated or non-glycosylated, may be compounded with other anti-viral
10   human milk components as well as other anti-viral and anti-microbial agents as indicated above. In another preferred embodiment, the product comprises the mucin complex or mixtures thereof. The polypeptide of this invention may be present in the anti-diarrheic product in an amount of about 0.01 to 99.9 wt% of the composition, and preferably about 0.1 to 20.0 wt%. However, other amounts of the agent may also be
15   present in the product. The amount of the agent in the anti-diarrheic product may be varied, and/or the frequency of administration increased, depending on the severity of the infection, the general health and nutritional status of the subject, and whether or not other anti-viral agents are being administered as well. Foodstuffs suitable for use in the anti-diarrheic product of the invention are milk, juices, cereals, chewing gum,
20   crackers, candies, meats, vegetables and fruits, blended or otherwise as baby food for example, and cookies, among others. In another embodiment, the foodstuff of the product provided herein may be infant formula, milk, milk substitutes, baby foods, rehydration formula, and vitamin supplements, among others. This product may be specifically formulated for the palate of youngsters, when applied to the treatment of
25   infants or small children.    The polypeptide may be provided in an anti-diarrheic kit comprising an anti-diarrheic composition comprising the polypeptide itself or a fragment thereof having anti-viral properties, either alone or with an agent selected from the group consisting of defatted human milk fat globules, skim milk, the human milk macromolecular fraction, curd, whey, the human milk mucin-70 Kdalton app. MW
30   glycoprotein-46 Kdalton app. MW HMFG antigen complex, other anti-diarrheic agents and additives mentioned above, and mixtures thereof, and a pharmaceutically acceptable carrier; and instructions for its use. The anti-diarrheic composition of this kit may be administered in an amount of the anti-diarrheic product of about 0.1 to 1000 mg/kg body weight/day, and more preferably about 1 to 50 mg/kg body
35   weight/day. Other amounts, however, may also be administered. It is understood that the more active fractions, such as the 46 Kdalton app. MW HMFG antigen may be

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administered at a lower dose, whereas the lesser active fractions such as the defatted milk fat globule may be administered at a higher dose. Other amounts may also be administered. This kit is formulated for the therapeutic treatment of subjects afflicted with or at risk of diarrheal conditions associated with viral infection. The additives for

5   the anti-diarrheic composition may be vitamin supplements, mineral additives, other nutritional additives, salts, buffers, flavoring compounds, diluents, thickeners, emulsifiers, preservatives, and anti-oxidants, among others, such as would be familiar to a person skilled in the art. Included within the invention, is an embodiment wherein the above anti-diarrheic compositions further comprise varying amounts of other

10  components such as foodstuffs. Suitable are all kinds of foods including milk and milk supplements. The anti-diarrheic composition or the product of the invention may also be modified to include varying amounts of water and ingredients suitable to the clinical needs of the subject. The anti-diarrheic composition may be mixed with a drink , soup, and the like (liquid) or a foodstuff (solid) for self-administration. The composition may

15  be added in an anti-viral amount, and may be provided in bulk or in unit form. An anti-diarrheic kit is also provided that comprises in separate, containers, a foodstuff, and an anti-viral effective amount of the polypeptide of the invention, either alone or with an agent selected from the group consisting of defatted human milk fat globules, skim milk, the human milk macromolecular fraction, curd, whey, the human milk mucin-70

20  Kdalton app. MW glycoprotein-46 Kdalton app. MW HMFG antigen complex, and mixtures thereof, and optionally a pharmaceutically-acceptable carrier, and instructions for use of the kit. For purposes of identification of the components, the apparent molecular weight (app. MW) of the glycoproteins of the invention may be determined by SDS-polyacrylamide gel electrophoresis using standard techniques described in the

25  art. For example, defatted human milk fat globule membranes may be dissolved in a solution containing 1% sodium dodecyl chloride (SDC) and heated to dissolve the glycoproteins, applied to a 3-30% polyacrylamide gel and electrophoresed with appropriate molecular weight standards run in a parallel lane, the apparent molecular weight (app. MW) of the mucin complex obtained is approximately 400,000 Kdalton

30  app. MW or greater. The apparent molecular weights of other proteins may be determined in a similar manner. The 46 Kdalton and the 70 Kdalton app. MW glycoproteins associated with the milk mucin complex may also be identified by binding to the specific monoclonal antibodies Mc16 and Mc13, respectively (Larocca et al., Cancer Res. 51:4994 (1991); Peterson et al., Hybridoma 9:221-235 (1990), supra).

35  The milk mucin, also referred to as breast mucin, may be identified in the complex by binding to the monoclonal antibody Mc5 described by Peterson, J.A., et al. (Peterson,

J.A., et al., Hybridoma (1990), supra). If the defatted human milk fat globule is dissolved in SDS under reducing conditions such as in the presence of 0.5% beta-mercaptoethanol, the 70 Kdalton app. MW HMFG glycoprotein runs as a doublet with an apparent molecular weight of 70 Kd, that may be further identified by binding to the

5  monoclonal antibodies Mc13 and McR2. The 46 Kdalton app. MW HMFG glycoprotein under the same conditions, appears as a doublet with an apparent molecular weight of 46 Kd, as identified by binding, among other antibodies evidencing specificity for different epitopes on the molecule, to the monoclonal antibodies Mc3 and Mc16 described by Larocca, et al. (Larocca et al., Cancer Res. 51:4994 (1991), supra). The

10  milk mucin, under reducing conditions, is seen as a band of approximate 400,000 Kdalton apparent molecular weight and may be identified by binding to the monoclonal antibody Mc5 described by Peterson, J.A., et al. (Peterson, J., et al., Hybridoma (1990), supra). If the milk mucin, the 70 Kdalton app. MW HMFG glycoprotein, and the 46 Kdalton app. MW HMFG glycoprotein are treated to remove oligosaccharides,

15  their apparent molecular weights, as determined by polyacrylamide gel electrophoresis, appear to decrease. This invention additionally provides a method for retarding the onset of, or countering, viral infection, such as that associated with rotaviruses, of a mammalian cell comprising contacting the cell in a nutrient medium with an anti-viral infection effective amount of the polypeptide of this invention or fragments thereof,

20  either alone or with an agent selected from the group consisting of defatted human milk fat globules, skim milk, the human milk macromolecular fraction, curd, whey, the human milk mucin-70 Kdalton app. MW glycoprotein-46 Kdalton app. MW HMFG glycoprotein complex, and mixtures thereof. In one preferred embodiment of the invention, the anti-diarrheic composition comprises the 46 Kdalton app. MW HMFG

25  glycoprotein. In another embodiment, the composition comprises the polypeptide of this invention and the mucin complex. Both of these agents may be administered alone and/or with defatted human milk fat globules, and/or whey, and/or curd, and/or skim milk, and/or the HMFG macromolecular component, and/or the 46 Kdalton app. MW HMFG glycoprotein, fragments thereof having anti-viral activity, and/or mixtures

30  thereof. Although the complete removal of glycosides from the mucin complex was shown to reduce the anti-viral activity of the glycoprotein by at least 40-60%, agents having varying levels of glycosylation may be used, since they retain some activity. Also disclosed herein is a method of retarding the onset of, or countering, viral infection of a subject's cells comprising administering to a subject at risk for, or

35  suffering from, a viral infection, such as a rotavirus infection an anti-viral effective amount of the composition of this invention or mixtures thereof, or a composition

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comprising the polypeptide of the invention and a pharmaceutically-acceptable carrier and/or a foodstuff and/or other additives as described above. The composition may incorporate other anti-viral or anti-microbial agents, as suitable for effective treatment of a rotavirus infection taking into account the age, general health, and nutritional

5    status of the subject. Other compositions of the agent of the invention and further comprising, e.g., the macromolecular fraction of the defatted milk fat globule membrane and the acidic fraction, are also contemplated herein.
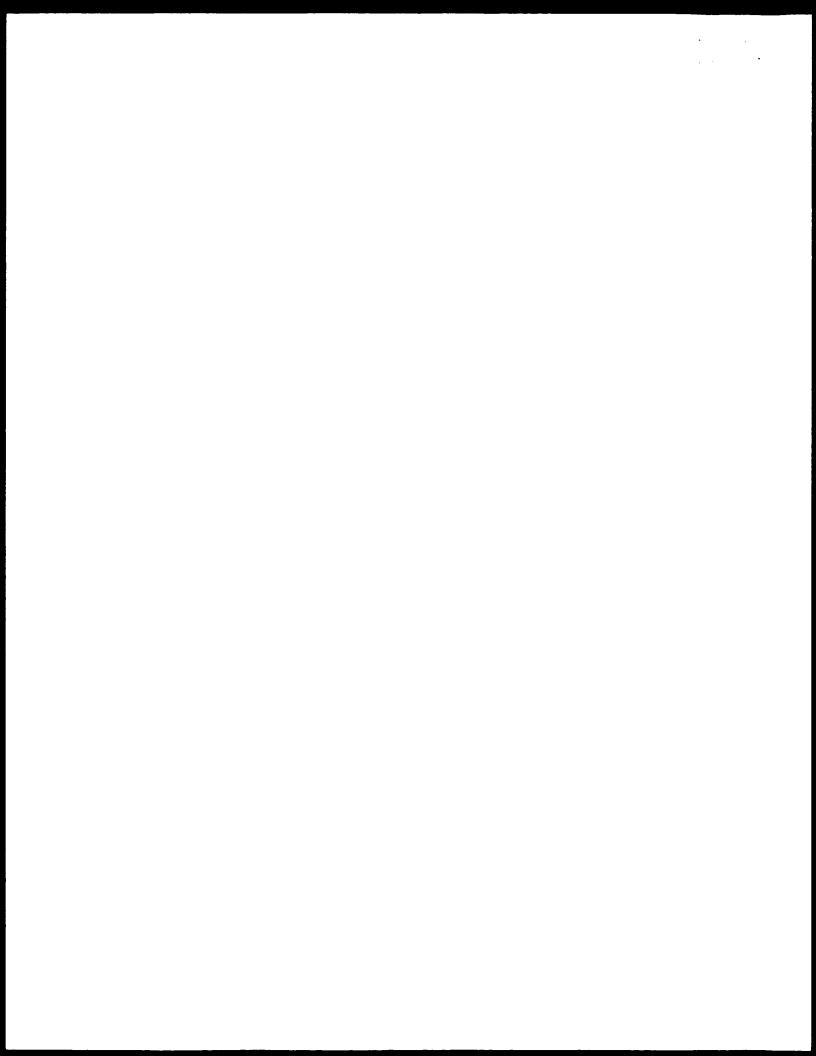
The onset of, or countering, infantile gasteroenteritis associated with viral infection, such as rotaviral infection, may be retarded or completely prevented by

10   administration to an infant or child in need of the treatment an anti-viral infection effective amount of the polypeptide of the invention, either alone or with an agent selected from the group consisting of defatted human milk fat globules, skim milk, the human milk macromolecular fraction, curd, whey, the human milk mucin-70 Kdalton app. MW glycoprotein-46 Kdalton app. MW HMFG antigen complex, and mixtures

15   thereof, and optionally a pharmaceutically-acceptable carrier and/or other agents and infant foodstuffs such as formula, milk, juice, and the like, as described above. The above method may be used for the prophylaxis of the disease, particularly where demographic and public health information suggests significant risk of infection. When symptoms indicate the onset of infection, the method may also be applied

20   therapeutically. The agent of this invention may be present in the infant formula in an amount from 0.01 to 99.9 wt%, and more preferably about 0.1 to 2.0 wt% of the composition. Other amounts of the agent, however, may also be used. As this product is formulated for the prophylactic or therapeutic treatment of infants and children afflicted with or at risk of diarrheal conditions associated with viral infection,

25   the infant food product may include varying amounts of infant formula, juices, foods, milk or milk supplements, among others. This anti-diarrheic infant product may also include vitamin supplements, water, mineral and other nutritional additives, salts, buffers, flavoring compounds, diluents, thickeners, emulsifiers, preservatives, encapsulation agents, glycosidase inhibitors, protease inhibitors, and anti-oxidants,

30   such as would be familiar to a person skilled in the art. The infant formula may also be modified to include varying amounts of water and other solutes to meet other clinical needs of the infant or child. The human milk components of this invention being routinely consumed and consisting of biological molecules, their administration will neither require clinical precautions nor medically trained personnel. Accordingly,

35   the present products may be sold over the counter. Also provided herein is a method of retarding the onset of, or countering, diarrhea associated with viral infections, such

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as rotavirus infection, in a subject's cells comprising administering to a subject in need of such treatment a composition comprising an anti-viral effective amount of the polypeptide of the invention, either alone or with an agent selected from the group consisting of defatted human milk fat globules, skim milk, the human milk

5    macromolecular fraction, curd, whey, the human milk mucin-70 Kdalton app. MW antigen-46 Kdalton app. MW HMFG antigen  complex, and mixtures thereof, and optionally a pharmaceutically-acceptable carrier and/or a foodstuff. Because of minimal side effects associated with the agent used in this method, the agent may also be administered for diarrheal symptoms regardless of etiology to prevent secondary

10   outbreaks associated with rotavirus infection. The polypeptide of the invention is also suitably applied to retard the onset of, or counter, diarrhea associated with viral infection in an immunodeficient subject comprising administering to an immunodeficient subject an anti-viral effective amount of the polypeptide, either alone or with an agent selected from the group consisting of defatted human milk fat globules, the human milk

15   macromolecular fraction, skim milk, curd, whey, the human milk mucin-70 Kdalton app. MW antigen-46 Kdalton app. MW HMFG antigen complex, and mixtures thereof, optionally comprising a pharmaceutically acceptable carrier and/or foodstuffs as described above. Such immunodeficiencies may result from genetic dysfunction, organ transplant, disease induced conditions or as a consequence of medical treatment with

20   drugs, among others. Other agents that may be added to the composition for this particular application are bulking agents, carbon black, high fiber additives, encapsulation agents, protease inhibitors, glycosidase inhibitors, and carrier lipids, optionally micellar, among others. These may be present in amounts known in the art. Specific applications of the above method are in cases of, e.g., transplants such as

25   bone marrow, kidney, heart and other organ transplants. Transplant patients receiving immunosuppressant drugs may also benefit from this anti-diarrheic treatment. The above preventative and therapeutic methods may be practiced by administering the agent provided herein as part of an anti-diarrheic composition also comprising a carrier or a product such as a foodstuff, as described above. Suitable foodstuffs are milk,

30   juices, cereals, powdered grains, candies, confections, cookies, meats, vegetables and fruits, put through a blender or otherwise processed, and crackers, among others.

A pharmaceutical or foodstuff composition for preventative, therapeutic, and/or imaging purposes may comprise the polypeptide of the invention and a non-proteolytic carrier. The carrier may be a pharmaceutically-acceptable carrier or in some cases a

35   foodstuff. This composition may be produced in bulk or in unit form. In the latter case, each unit may contain an antibody binding effective amount (in vitro and ex vivo

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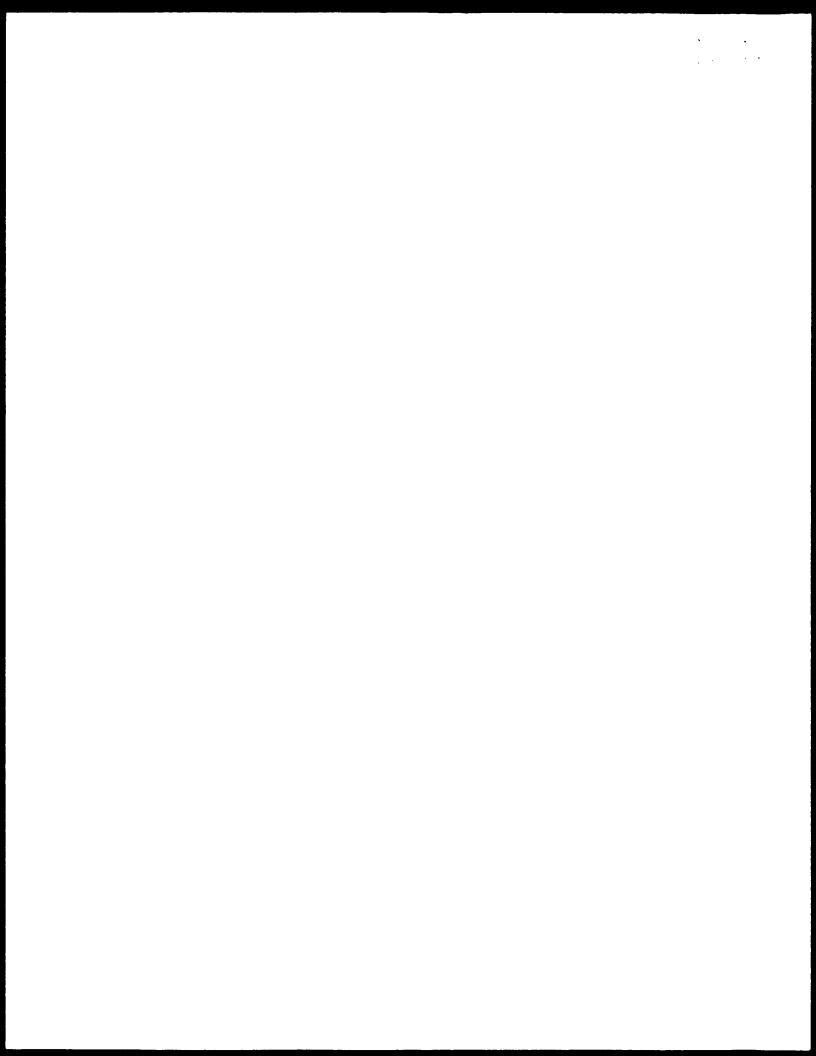
assays), an anti-viral effective amount (anti-viral therapy), or an anti-cancer therapeutic amount (cancer therapy) of the polypeptide. The pharmaceutical or foodstuff composition is intended for in vivo animal use, which includes human administration. Each dose preferably contains about 0.1 to 1000 mg of the polypeptide per kg body

5　weight, and more preferably about 10 to 500 mg/kg. However, other amounts may also be administered as a practitioner would know. Any pharmaceutically-acceptable carrier may be utilized for the preparation of the composition intended for in vivo therapy or diagnostic use. Examples of suitable carriers and other additives are flavorings, preservatives, bulking materials, stabilizers, adjuvants, coatings, colorants,

10　and salt solutions such as saline, oils or solids, among others as known in the art. However, any liquid or solid carrier which does not hydrolyze the polypeptide is suitable particularly for in vitro and ex vivo uses. The pharmaceutical or foodstuff composition as well as the polypeptide itself are best kept under refrigeration and/or frozen as is known in the art. The polypeptide and the pharmaceutical or foodstuff composition

15　may be vacuum dried and packaged in a sterile container for transportation to their destination. The composition may comprise about 0.01-99.99 wt% of the polypeptide, and preferably about 0.1-10 wt%, the remainder being the carrier when the composition is intended for in vitro application only, in which case the carrier need only be non-proteolytic. Also provided herein is a fusion protein, which comprises the

20　polypeptide described above, and a second antigenic polypeptide or an antibody binding fragment thereof which is operatively bound to the polypeptide. The polypeptide of the invention may be bound to a fragment of the second antigenic polypeptide as peptides about 10 to 1000 amino acids long and 10 to 1100 amino acids long, respectively, and preferably about 15 to 300 amino acids long and 200 to 400 amino

25　acids long, respectively. However, other sizes of the polypeptides, and/or fragments thereof, either larger or smaller, may be utilized as long as their antibody binding capability is preserved. Any polypeptide is suitable as the second antigenic polypeptide as long as it acts as an antigen to elicit the formation of antibodies by a mammal when intended for use in a multiple antibody assay. The second antigenic polypeptide may

30　also be chosen by some other property suitable for the identification and/or use of the fusion protein, such as a function other than antigenicity. By means of example, the second antigenic polypeptide may be a protein such as ß-galactosidase or a fragment thereof. Both, the polypeptide of the invention and the fusion protein may be prepared by methods known in the art, either synthetically or by expression of a DNA fragment

35　that encodes it as described herein or by cloning and expression utilizing other methods known in the art. The fusion protein may be prepared, for instance, by cloning a

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recombinant DNA encoding, in reading frame, the gene's segment into a vector carrying the DNA encoding the second polypeptide, transfecting a suitable host, and expressing it in a host.

Also part of this invention is an antibody having high affinity, selectivity and
5   specificity for the 46 Kdalton  HMFG antigen of the invention or fragments thereof containing one or more of its epitopes.  These antibodies are of greater specificity for cancer cells than the original antibodies used to isolate the mRNA because they identified epitopes on the polypeptide that are probably more accessible to the antibody when the polypeptide is on the cell.  Monoclonal antibodies Mc8 and Mc 16 bind to the
10  C1C2 domain of the 46 Kdalton  HMFG antigen, that is considered to be the domain most likely buried in the cell membrane.  Also antibodies against the cell adhesion sequence RGD and the EGF-like sequence are intended for modifying the function of the 46 Kdalton  HMFG antigen in cancer cells and thus diminish the cancerous properties of these cells.  Also, the antibodies that bind to more accessible epitopes
15  evidence greater applicability in immunohistochemistry. Methods for raising antibodies are known in the art and need not be described herein.  For instance, the amino acid sequence corresponding to a desired epitope may be utilized as a hapten or antigen and with the aid of a carrier protein and adjuvants administered to an animal to raise epitope-specific antibodies.  The B-cells producing these antibodies may then be utilized
20  to produce hybridomas by methods known in the art that express highly specific antibodies for selected epitope.  Particularly preferred are monoclonal antibodies.  The antibodies raised against the biologically pure polypeptide or epitopic fragments thereof have increased affinity and/or specificity for the polypeptide.  Typically, the affinity constant may be about $10^8$ to $10^5$ $M^{-1}$, and in some cases greater than $10^8$ $M_{-1}$.
25  Particularly preferred embodiments of the antibody also have affinity for the C and/or C2 regions of clotting factor VIII (light chain) and the RGD and/ or EGF-like segments thereof.  Still another preferred antibody of the invention are binding active Fab, $(Fab)_2$, and Fab' fragments thereof.  Also preferred are binding active single chains of the antibody or the fragments.  A composition intended for in vitro use comprises an anti-
30  46 Kdalton HMFG antigen antibody having an affinity constant of about $10^{10}$ to $10^5$ $M^{-1}$, or binding fragments thereof, and a non-proteolytic carrier.  When intended for in vivo or ex vivo use, the carrier must be a pharmaceutically-acceptable carrier or a foodstuff.  When in unit dose form, the antibody is typically provided in an amount of about 0.001 to 100,000 mg, and more preferably about 10 to 500 mg.  However,
35  other amounts are suitable.  Any pharmaceutically-acceptable carrier is suitable as indicated above.  Other ingredients may also be contained in the composition such as

radionuclides, chemotherapeutic drugs, interferon, toxic agents such as ricin A-chain, abrin A-chain, saline salt solutions, preservatives, flavors, bulking agents, colorants and buffers, among others, as is known in the art. The preparation of all the compositions may be undertaken by admixing the polypeptide or the antibody with the

5    pharmaceutically-acceptable carrier under non-proteolytic conditions, then vacuum dried and packaged in sterile containers or provided as a sterile solution.

The present antibodies may be applied to detecting the presence in a biological sample of the 46 Kdalton  HMFG antigen or fragments thereof, by addition to a biological sample suspected of containing the polypeptide, adding thereto an antibody

10   selectively binding the 46 Kdalton  HMFG antigen under conditions effective to form an antibody-polypeptide complex, determining the amount of complex formed, and preparing the result with a control run without the sample. This method is suitable for detecting the presence of the polypeptide or fragments thereof in biological samples such as animal cells, cell extracts, body fluids such as milk, and aids in the

15   determination of whether epithelial cells or neoplastic tumor cells such as those from breast and other tissues which are of epithelial origin and express the 46 Kdalton HMFG antigen, are present in the sample. Typically, all body fluids are encompassed herein. Examples are serum, plasma, urine, breast fluid, human milk, tissue biopsies, and fine needle aspirates. The sample may be previously treated, e.g., to avoid

20   interference by metals, non-specific proteins, fats, nucleic acids, and the like as is known in the art. The biological sample may also be diluted in order that the protein content be in a range of about 0.0001 to 10 mg/ml, and more preferably about 0.001 to 0.1 mg/ml. The antibody may be added in an amount of about 0.0001 to 1.0 mg/ml of sample, and more preferably about 0.001 to 0.1 mg/ml of sample. Other conditions

25   for the assay utilized, including the following. The sample may be homogenized and centrifuged to remove particulate material and fatty material. Detergents may be added to dissolve membranes, solubilize fatty material and reduce background. Also added may be carrier proteins such as bovine serum albumin to reduce non-specific binding of the antibodies, and chelators to remove interfering divalent metal ions. The

30   antibody may be monoclonal or polyclonal, although preferred are monoclonal antibodies which provide high sensitivity. Even more preferred are antibodies of affinity constants of about $10^8$ and up to about $10^{10}$ $M^{-1}$. The determination of the presence of any complex formed between the antibody and the polypeptide may be done by a variety of methods known in the art. By means of example will be cited

35   herein the further addition of a labeled anti-constant region immunoglobulin to form a labeled double antibody-polypeptide complex. The label may be a radiolabel, a

26
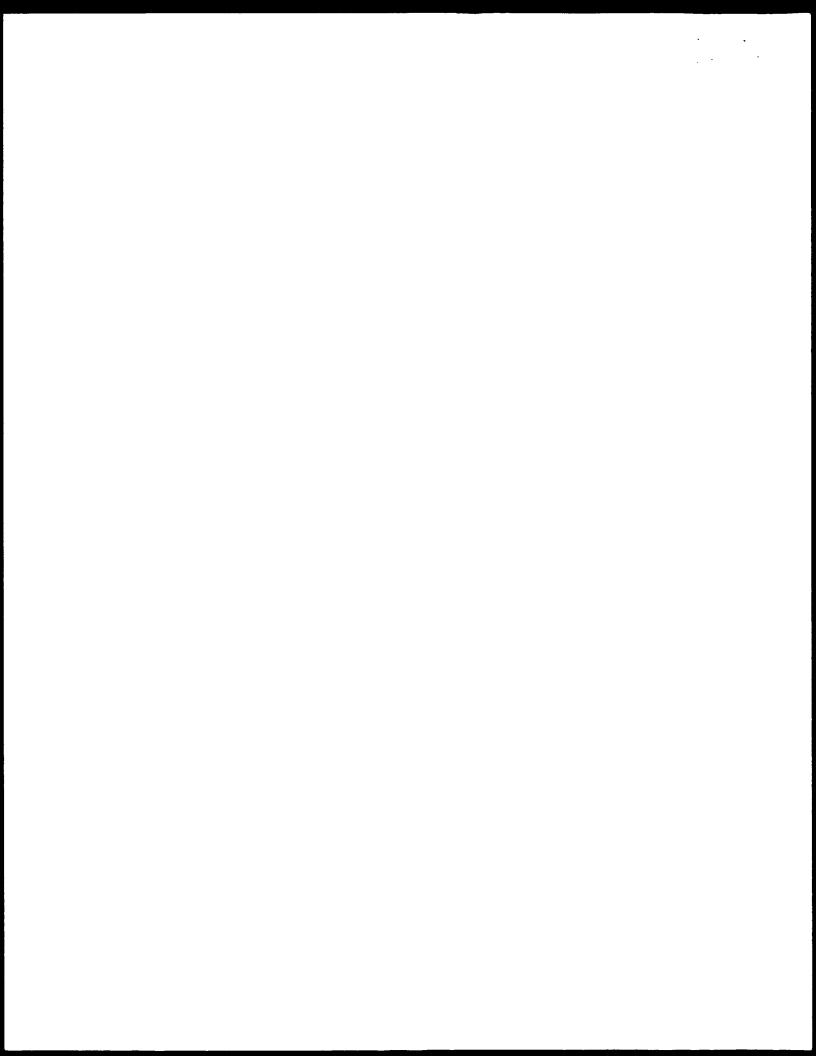
fluorescent label, an enzyme label or biotin to be later detected as a conjugate of avidin, streptavidin or magnetic bead, among others. After this step, the amount of label bound to the complex may be assessed by methods known in the art. This method may be applied to determining the presence in a biological sample of epithelial

5   cells or the 46 Kdalton HMFG antigen itself or a fragment thereof by adding to a biological sample suspected of containing cells of epithelial origin or the antigen or a fragment thereof such as cancer patient's serum samples an anti-46 Kdalton HMFG antigen antibody, and determining the amount of any complex formed therebetween. This method is particularly well suited for biological samples such as bone marrow, milk

10  and serum samples. However, it may be practiced with samples of other origins as well. The steps are in general conducted as described above and the determination of the presence of malignant tumor cells of epithelial origin or anti-viral factors in milk may be done by the identification, either qualitative or quantitative, of any complex formed with the antibody as already described. The detection may also be undertaken by

15  assaying for the presence of ribonucleic acid (RNA) encoding the 46 Kdalton HMFG antigen using nucleic acid probes based on sequences such as the ones shown in Table 1 and 4 or fragments thereof, and methods known in the art such as PCR (Erlich, H.A., in PCR Technology: Principles and Applications for DNA Amplification, Stockton Press (1989)).

20          The antibody may also be applied to the in vivo imaging or therapy of malignant tumors of epithelial origin by administering to a subject suspected of being afflicted by a cancer of epithelial origin, or undergoing cancer therapy, a polypeptide binding effective amount of an anti-46 Kdalton HMFG antigen antibody of this invention effective to deliver it to an area of the subject's body suspected of having the

25  neoplastic tumor to form an antibody-cell polypeptide complex. The antibody may carry a radiolabel or other anti-cancer therapeutic agent or a detectable label capable of binding to the antibody at a site other than polypeptide binding site may be administered, and then non-invasively detecting the presence of the label associated with any complex formed in the subject's body. The antibody may be administered at

30  a concentration of about 0.5 to 50 mg/ml, and more preferably about 5 to 20 mg/ml. A total of about 1 to 50 ml of the antibody composition may be given at any one particular time. The regimen of administration may be by single or repeated dosage, or the antibody may be administered in a continuous manner in order to image or to continuously suppress the presence of tumor cells. The antibody may be administered

35  in a pharmaceutical composition as described above, or in any other suitable form. The administration of the antibody may be conducted by intravenous, intraperitoneal,

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intracavitary, lymphatic, intratumor or intramuscular routes, among others. Other routes are also suitable if they do not hydrolyze the peptide links of the antibody. The administration of a detectable label may be conducted by utilizing a labeled anti-constant region immunoglobulin, protein G or A or a binding fragment thereof, and then
5    detecting the amount of label bound to the complex. These technologies are known in the art and need not be further described herein.

The polypeptide and fusion protein of the invention may be applied to detecting the presence in a biological sample of anti-46 Kdalton HMFG antigen antibodies, indicative of a growing neoplastic tumor of epithelial origin such as a carcinoma, by
10    adding to a sample obtained from a patient suspected to be afflicted with this type of cancer an antibody binding effective amount of the polypeptide of the invention and determining the presence of any complex formed. The sample may be treated as indicated above to eliminate interference by other proteins and/or components present in the sample. In the case of blood, serum may be obtained first, and then the serum
15    may be treated by adding normal human or bovine serum, and/or bovine serum albumin (BSA) is used as a blocking agent to reduce non-specific reactivity. The polypeptide may be recombinantly produced and is typically added to the sample in an amount of about 0.00001 to 1.0 mg/ml sample, and more preferably about 0.0001 to 0.1 mg/ml sample. However, other amounts may also be utilized as seen for different assay
20    procedures, and the amount of antibody in the sample may be controlled by dilution. Optimal ranges of antibody in the sample are about 0.00001 to 0.1 mg/ml, and more preferably about 0.0001 to 0.01 mg/ml, but, other amounts may also be utilized. The steps of this method are practiced as described above, including the determination of the presence of antibody-polypeptide complex. The conditions for the assay are in
25    general those known in the art for not denaturing proteins and the overall variables, such as pH, temperature and the like may be adjusted without undue experimentation. The presence of an anti-46 Kdalton HMFG antigen antibody in a sample may also be detected by adding to a sample suspected of comprising the antibody a binding effective amount of the fusion protein of this invention under conditions effective to
30    form an antibody-fusion protein complex, adding thereto an anti-second polypeptide antibody under conditions effective to form a double antibody complex, and determining the presence of any double antibody complex formed. This method is preferably practiced with an anti-second polypeptide monoclonal antibody. The amount of anti-second polypeptide antibody added to the sample is preferably about 0.00001
35    to 0.1 mg/ml sample, and more preferably about 0.0001 to 0.01 mg/ml of sample. However, other amounts may also be utilized, and the sample may be pretreated prior

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to the addition of the fusion protein in various manners, such as by dilution and/or elimination of interfering components. These steps are undertaken as is known in the art and need not be further described herein. Solid-phase type of assays are preferred, and among these, more preferred is the Ceriani et al. method (Ceriani, R.L. et al., Anal.

5    Biochem. 201:78 (1992)). However, other assays are also suitable and therefor, contemplated herein.

The polypeptide of the invention is also useful for vaccinating against neoplastic tumors and cancer by its administration or that of antigenic fragments thereof in amounts effective to elicit an endogenous immunological response. This in vivo

10   method may also be utilized in cancer patients to induce an immune response against their exposed neoplastic epithelial cells carrying the corresponding epitopes. The vaccinating polypeptide may be administered to a subject including a human in an amount of about 0.1 to 100 mg/ml, and more preferably about 2 to 50 mg/ml. Typically, any dose may be delivered in about 0.1 to 50 ml, and more preferably in

15   about 1 to 10 ml of the carrier. The vaccinating composition may be administrated in a single dose or it may be administered repeatedly and/or on a continuous basis for periods of up to about 6 months, and sometimes in excess of one year, alone with a carrier or in conjunction with one or more adjuvants, and the like, as is known in the art. More prolonged periods of time are also encompassed for vaccination according

20   to this invention.
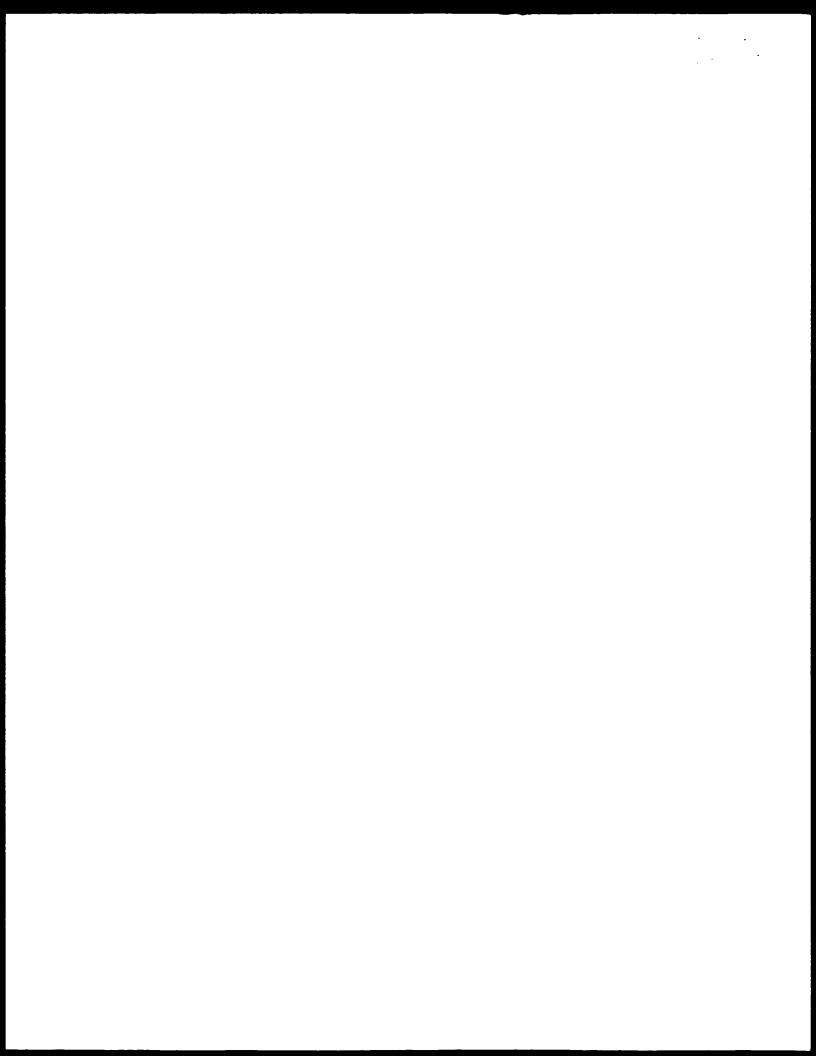
A therapeutic agent may be delivered in vivo to target epithelial cells, such as neoplastic cells by binding it to the anti-46 Kdalton HMFG antigen monoclonal antibody provided herein at a site other than the antigen binding site, administering to a subject afflicted with a neoplastic growth of epithelial origin a therapeutically

25   effective amount of the antibody-bound therapeutic agent under conditions effective to deliver the agent to the target cells environment, and allowing the antibody carrying the therapeutic agent to bind to the target cells to permit the therapeutic agent to exert its effect on the cells. This in vivo method may be utilized for treating cancer patients that are afflicted with cancers of epithelial origin, e.g. breast cancer. The therapeutic

30   agent may be any anti-cancer agent known in the art, such as radionuclides, chemotherapy drugs, toxic agents such as ricin A-chain, abrin A-chain, and others. The therapeutic agent is typically bound to the antibody by means known in the art. More specifically, a radionucleide such as $^{131}$I may be bound to the antibody by oxidation of amino acids such as tyrosine, or $^{90}$Y may be attached via a chelator, and

35   the conjugate injected intravenously or intraperitoneally into humans afflicted with neoplastic tumors such as breast carcinomas among others to inhibit the growth of the

tumor. (e.g., for mice, Ceriani, et al, Cancer Res. 48:4664-4672(1988)). The antibody-bound therapeutic agent may be administered to the subject in an amount of about 1 to 100 mg/ml, and more preferably about 2 to 20 mg/ml. Typically, any dose will consist of about 1 to 50 ml of carrier and more preferably about 2 to 10 ml carrier.

5    The antibody-bound therapeutic agent may be administered as a single dose, in multiple doses, or on a continuous basis for periods of up to about 6 months, and sometimes in excess of one year.   More prolonged periods of time are also encompassed for treatment herein.

The therapeutic agent may also be delivered ex vivo to target cells such as

10   neoplastic tumor cells by adding the antibody-bound therapeutic agent to a sample obtained from a patient afflicted with cancer under conditions effective to promote the formation of an antibody-cell polypeptide complex, allowing the agent to exert its effect on the cells, and returning the sample to the subject.  Non-conjugated antibody may also be added to the sample in the presence of complement, which causes lysis

15   of the cells, prior to returning the sample to the subject.  In general, the steps of this method may be practiced as described above for other applications, particularly in terms of the preparation of the biological sample, and binding of the therapeutic agent to the antibody as well as the addition of the antibody-bound therapeutic agent to the sample. The sample may be returned to the subject by means known in the art.  For

20   example, the already treated sample may be returned to a subject's body in sterile form by the intravenously, intracavitary, intraperitoneal, and intratumor routes, among others.  However, other routes known in the art may also be utilized.

Also provided herein is a polynucleotide encoding the polypeptide of this invention including all redundant DNA and RNA sequences.  The polynucleotide is

25   provided either as a double stranded or single stranded DNA containing the coding or the non-coding strand of the polynucleotide. The fragments of the polynucleotide may be of about 15 to 3000 bases, and more preferably about 30 to 300 bases. Both the double stranded and the single stranded DNAs discussed above may be in labeled form. The labeling may be conducted as is known in the art with radioactive atoms such as

30   $^{32}P$, $^{14}C$, $^{3}H$, $^{33}P$, and the like.  However, other radionuclides may also be utilized. Particularly preferred is a polynucleotide encompassing the DNA sequence shown in Tables 1 and 4 of this patent and redundant sequences thereof encoding the polypeptide of the invention or fragments thereof comprising about 9 to 3000 bases, and more preferably about 18 to 300 bases. However, fragments of other sizes may

35   also be utilized and are encompassed herein. Also part of this invention is a polyribonucleotide encoding the polypeptide of the invention or fragments thereof.  The

polyribonucleotide segments may be about 9 to 3000 bases long, and more preferably about 18 to 300 bases long. However, other fragment sizes are also encompassed herein. Still part of this invention is a non-coding strand of a polyribonucleotide having a sequence complementary to that of the polyribonucleotide described above. This

5  polyribonucleotide sequence is capable of hybridization to the coding RNA strand or to the non-coding strand of the corresponding DNA. In a particularly preferred embodiment the polyribonucleotide is provided in labeled form.

A hybrid polynucleotide encoding a fusion protein comprising the above polypeptide and a second antigenic polypeptide or antibody binding functional fragment

10  thereof bound thereto. The hybrid polynucleotide may be about 15 to 4000 bases long, and sometimes longer, and more preferably about 50 to 1,800 bases long. However, other size polynucleotides are also encompassed herein. Also provided herein is a hybrid polyribonucleotide encoding a fusion protein comprising the polypeptide of the invention and a second antigenic polypeptide, or antibody binding

15  fragment thereof bound thereto and all redundant fragments thereof and complementary sequences thereof. The hybrid polyribonucleotide encoding the fusion protein may be about 15 to 4000 bases long, and more preferably about 50 to 1,800 bases long. Fragments thereof may be about 9 to 100 long, and more preferably about 15 to 70 bases long. The hybrid polynucleotide encoding the fusion protein is provided

20  as a double stranded DNA which encompasses the coding or non-coding strand encoding the fusion protein or fragments thereof. The latter polynucleotide provided herein is a polynucleotide comprising DNA sequences complementary to the polynucleotide encoding the fusion protein. Both the DNA and RNA sequences encoding the fusion protein may be provided in labeled form. Particularly useful labels

25  are $^{32}P$ and others known in the art. The DNAs and RNAs are labeled by methods known in the art.

The presence of a polynucleotide encoding the 46 Kdalton HMFG antigen or a fragment thereof in a sample may be detected by adding to the sample a hybridization effective amount of a labeled DNA comprising the non-coding strand of a

30  polynucleotide encoding the polypeptide or hybrid polypeptide of the invention under stringent conditions effective to hybridize any polynucleotide having a complementary sequence of at least 15 bases thereto, and detecting the presence of the DNA-complementary polynucleotide hybrid. The sample may be a biological sample or it may be a laboratory sample. If the sample contains cells where the polynucleotide is

35  located, the cells may need to be lysed, and optionally the DNA isolated from the remainder materials. This may be done by methods known in the art. The sample may

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be diluted and/or otherwise prepared for the melting of double stranded polynucleotide sequences present therein. The melting step is conducted as is known in the art. In general, the sample is prepared by lysing the cells in 4 M guanidinium isothiocyanate to denature protein and prevent RNAse activity. Extracts are run on a cesium chloride

5 density step gradient ultracentrifugation where RNA, DNA and protein are separated according to their relative densities. DNA and RNA may be further purified by extraction with organic solvents, and concentrated by precipitation in 70% ethanol. (Sambrook et al, in Molecular Cloning: A Laboratory Manual, Second edition, Cold Spring Harbor Press, N.Y., (1989)). Melting may be accomplished by raising the

10 temperature of the sample about 20°C over the Tm of the DNA, or by raising the pH to above 12. To the melted DNA may be added a hybridization effective amount of the labeled non-coding DNA strand. Suitable conditions for the hybridization of DNA-DNA segments are known in the art. The degree of stringency is determined by the degree of complementarity of the sequences desired to be hybridized. In general, when more

15 stringent conditions are utilized hybridization will occur only with DNA sequences which have a high degree of complementarily with the probe. Thus, a low degree of stringency is desired to detect sequences with low complementarity, and the conditions may be varied accordingly. In general, the conditions may be as follows. The sodium ion concentration is about 1M, the pH about 5-9, the temperature about

20 65°C or about 20°C below the melting temperature of the duplex DNA of the probe sequence and its complementary strand (Britten, R. et al, Methods in Enzymology 29:363(1974); Sambrook et al, supra). The DNA-complementary polynucleotide labeled hybrid may be detected by methods known in the art. Typically, the double stranded DNA is restricted with enzymes and electrophoresed on a gel to separate the

25 different size fragments. The gel is blotted onto a specially prepared filter, hybridized, and the filter is then exposed to a photographic plate for an effective period of time. The plate is then developed and the different fragments analyzed. For a more qualitative detection of the presence of the double stranded labeled hybrid, the unrestricted DNA may be blotted onto a filter, hybridized, exposed to a photographic

30 plate and the plate developed to merely detect the presence of radiolabel.

The presence of an RNA sequence encoding the 46 Kdalton HMFG antigen or a fragment thereof may be determined by adding to a sample suspected of containing the RNA, a hybridization effective amount of the coding strand of a labeled polynucleotide encoding the 46 Kdalton HMFG hybrid polypeptide or fragment thereof

35 antigen in single stranded form under stringent conditions effective to hybridize any RNA having a complementary sequence of about at least 15 bases thereto, and

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detecting the presence of the polynucleotide-RNA hybrid. In essence, this method is conducted as previously described for detection of a DNA sequence, with the additional precaution of substantially ensuring the absence or RNAses in the mixture. In general, the following must be additionally done when detecting RNA. The use of RNAase

5    inhibitors and the pretreatment of labware with diethylpyrocarbonate to inactivate any contaminating RNAase. Hybridizations are conducted generally at a higher stringency because RNA:RNA hybrids are more stable than DNA:DNA hybrids. For example, the hybridization may be conducted at 65°C in 50% formamide. The Tm of DNA duplexes is reduced by about 0.72°C per 1% formamide added. (See, Sambrook et al, supra;

10   Casey J. and Davidson N., Nucl. Acids Res. 4:1539-1552(1977)). If the RNA is contained inside the cells, the cells must be lysed to expose the ribonucleic acid. This is done by means known in the art such as detergent lysis, which may be followed by treatment with proteases.

Also part of this invention is a DNA segment comprising an anti-sense

15   polynucleotide to the coding strand of the polynucleotide of the invention of about 200 to 1,800 nucleotides. More preferably, the DNA segment may have about 100 to 1,000 nucleotides. The concept of anti-sense sequences is generally known in the art. Synthetic oligonucleotides may be prepared that are complementary to the messenger RNA encoding a target protein. The oligonucleotide or a chemically modified equivalent

20   thereof are then added to cells. The oligonucleotide binds the target mRNA and thus inhibits the translation of the target protein. (Markus-Sekura C.J., "Techniques for using Antisense Oligonucleotides to Study Gene Expression", Analytical Biochemistry 172:289-295(1988)). Alternatively, antisense-RNA may be used to block translation of sense RNA. The antisense RNA may be generated from a viral or plasmid DNA

25   vector that contains a copy of the target gene situated in the reverse orientation with respect to the direction of transcription. A virus may be used as a carrier to introduce the inverted gene into the target cell genome. (Izant, J.G. and Weintmub H., Science 229:345-352(1985)). Fragments of the anti-sense DNA segment are also provided herein and they may comprise about 15 to 100 bases, and more preferably 30 to 50

30   bases. The anti-sense sequences may be obtained by methods known in the art such as the following. Antisense oligonucleotides can be made by modifying their phosphate moiety to increase biological lifetime, to enhance the permeability of the cells and to strengthen binding to the target. For example, oligomethylphosphonates (Miller, P.S., Reddy, M.P., Murakami, A., Blake, K.R., Lin, S.B. and Agris, C.H. (1986)

35   Biochemistry 25:5092-5097), or oligophosphorothionates (LaPlanche, L.A., James, T.L., Powell, C., Wilson, W.D., Uznanski, B., Stec., W.J., Summers, M.F. and Zon, G.

(1986) Nucleic Acids Res. 14:9081-9093). Alternatively, the target gene may be inserted into a viral-based eukaryotic expression vector in reverse orientation and introduced into mammalian cells (See, Sambrook, J. et al, supra). The anti-sense DNA may be provided as a pharmaceutical composition which comprises in addition to the

5    anti-sense DNA or fragment thereof, a pharmaceutically-acceptable carrier. The composition may comprise different amounts of the components. Typically, the anti-sense DNA is contained in an amount of about 0.01 to 99.99 wt%, and more preferably about 0.1 to 20 wt% of the composition, the remainder being carrier and/or other known additives. The pharmaceutically-acceptable carrier may be any carrier

10   which does not degrade DNA and is physiologically tolerated. Examples of carriers and other additives are sterile buffered saline solution, human serum albumin and the like. However, others may also be utilized. The pharmaceutical composition may be prepared by admixing the anti-sense DNA with the carrier and other components as is known in the art, freeze dried and packaged in a sterile container. The composition

15   may be maintained refrigerated and/or frozen. The anti-sense product may be applied to the treatment of cancer of epithelial origin by administering it as a composition comprising a therapeutically effective amount of the anti-sense DNA segment of this invention or a fragment thereof. This method may be practiced by administering about 5 to 800 mg anti-sense DNA per day, and more preferably about 20 to 200 mg anti-

20   sense DNA per day in a pharmaceutical composition. The composition may be administered by a parenteral, intravenous, intracavitary or other localized route. However, other routes of administration may also be utilized.

        Part of this invention is also an immunoassay kit comprising, in separate containers, a monoclonal antibody having specificity for the 46 Kdalton HMFG antigen

25   of this invention or Fab, (Fab)$_2$, or Fab' fragments thereof, anti-constant region immunoglobulin, protein G or A or binding fragments thereof for use with entire antibodies, and instructions for its use. This immunoassay kit may be utilized for practicing various of the methods provided herein. The monoclonal antibody and the anti-constant region immunoglobulin or other antibody binding molecules may be

30   provided in amounts of about 0.001 mg to 100 grams, and more preferably about 0.01 mg to 1 gram. The anti-constant region immunoglobulin and other antibody binding molecules may be a polyclonal immunoglobulin, protein A or protein G or functional fragments thereof, which may be labeled prior to use by methods known in the art. The antibody may also be provided as an immunotherapy kit, comprising in addition,

35   in separate containers, a therapeutic agent such as an anti-cancer agent and instructions for using the kit and for attaching either the therapeutic agent or a

radiolabel to the antibody or to a further component such as immunoglobulin, protein G or A or binding fragments thereof.
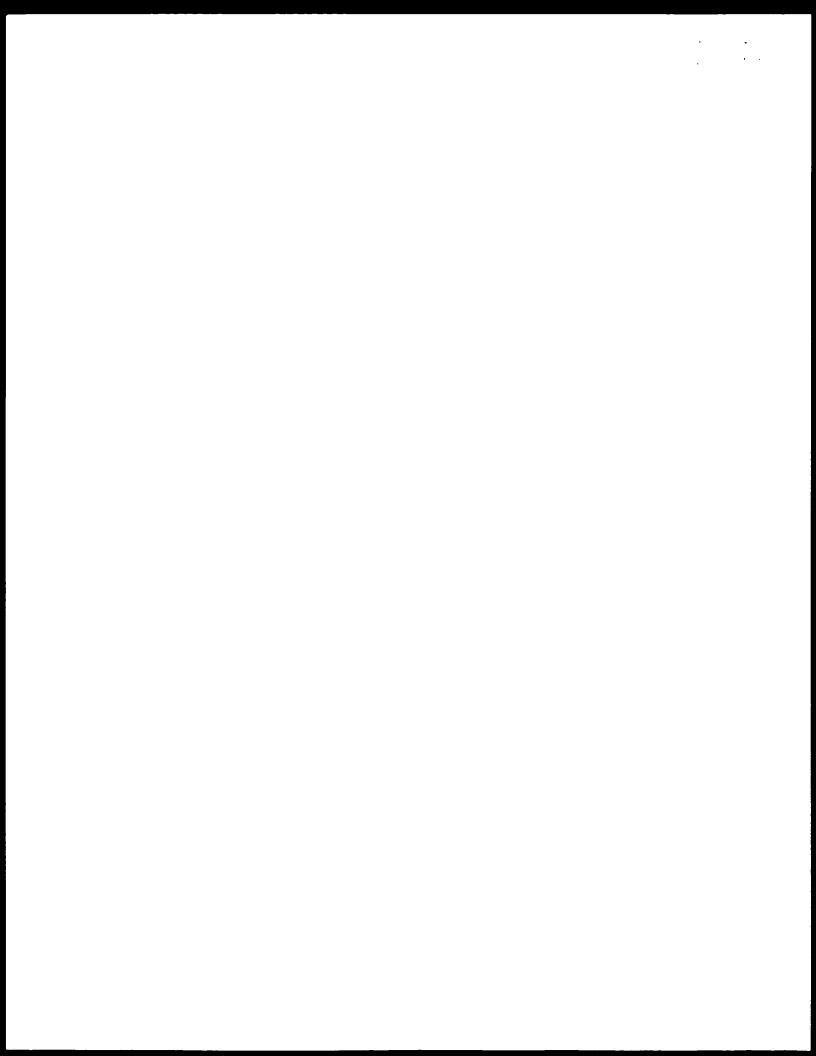
Also provided herein is an antibody detecting kit comprising, in separate containers, a polypeptide having the antibody binding specificity of the 46 Kdalton

5   HMFG antigen, anti-constant region immunoglobulin, protein G or A or fragments thereof, and instructions for its use. The polypeptide may be a recombinantly obtained peptide and the anti-antibody immunoglobulin may be labeled prior to use. A fusion protein kit comprises, in separate containers, the fusion protein of this invention, an anti-second polypeptide monoclonal antibody, anti-constant region immunoglobulin,

10  protein G or A or binding fragments thereof, and instructions for its use. The fusion protein may be provided in sterile form in an amount of about 0.001 mg to 100 grams, and more preferably about 0.01 mg to 1 gram. The anti-second polypeptide monoclonal antibody may also be provided in sterile form in an amount of about 0.001 mg to 100 grams, and more preferably about 0.01 mg to 1 gram. The anti-constant

15  region immunoglobulin, protein G or A or fragments thereof may be provided in a separate sterile container in an amount of about 0.001 mg to 100 grams, and more preferably about 0.01 mg to 1 gram. The entire kit may be packaged for shipping and storage. An anti-cancer therapeutic kit provided according to this invention comprises, in separate containers, a monoclonal antibody selectively binding the 46 Kdalton

20  HMFG antigen, an anti-cancer agent selected from the group consisting of immunotoxins and radionuclides and instructions for its use. The monoclonal antibody may be provided in sterile form in an amount of about 1 mg to 20 grams, and more preferably about 2 mg to 10 grams. The antibody may be freeze-dried and packaged and the therapeutic agent may be any known anti-cancer agent. By means of

25  example, the agent may be abrin-A chain, ricin A-chain, immunotoxins, chemotherapy drugs, and $^{131}$I and $^{90}$Y radionuclides, among others.

Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein for purposes of illustration only and are not intended to be limiting of the invention or any

30  embodiment thereof, unless so specified.

## EXAMPLES

Example 1:            Immunoscreening λgt11 cDNA library

Two human breast cDNA libraries were purchased from Clontech (Palo Alto, CA). The first library was originally prepared from RNA extracted from adult breast

35  tissue excised during mastectomy obtained during the 8th month of pregnancy and

showing well-differentiated tissue and lactational competence. The other cDNA library, ZR75, was reverse transcribed from mRNA extracted from the breast carcinoma cell line ZR75. The oligo-dT primed cDNA from this tissue was inserted into the Eco R1 site of λgt11. Plating and screening of the library with monoclonal antibodies was

5    done essentially as described by Young and Davis (Young, R.A. and Davis, R.W., PNAS (U.S.A) 80:1194-1198 (1983)). The library was screened with a cocktail of monoclonal antibodies Mc3, Mc8, Mc15 and Mc16 all of which bind the 46 Kdalton component of human milk fat globule. (Peterson et al, Hybridoma (1990), supra; Larocca et al, Cancer Res. 51:4994 (1991)).

10   **Example 2:**          **Blot Analysis**

The cell lines were grown to late log phase and total cell RNA prepared by the method of Chirgwin et al. (Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. Biochemistry 18:5294-5299. (1979)). RNA was glyoxalated, electrophoresed, and blotted according to Thomas (Thomas, P., "Hybridization of

15   denatured RNA and small DNA fragments transferred to nitrocellulose", PNAS (USA) 77:5201-5205 (1980)) and RNA bound to nylon (Biodyne) filters using UV irradiation.

Single stranded RNA probes were made in vitro using SP6 and T7 RNA polymerase according to manufacturer (Promega) and labelled by incorporation of $^{32}$P-UTP at 800 Ci/mmol (Amersham). Hybridization of RNA probes to RNA blots was at

20   70°C, 0.1 x SSC, 0.1% SDS. Blots were exposed to X-ray film (Kodak X-AR) at -80°C with intensifying screens.

**Example 3:**          **DNA Sequencing**

Large scale bacteriophage DNA preparations were made from phage lysates, and the Eco R1 digested cDNA insert subcloned into pGEM3 (Promega, Madison, WI)

25   according to standard protocols (Sambrook, J., Fritsch, D., and Maniatis, T., in Molecular Cloning: A Laboratory Manual/Second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1990)). Dideoxy sequencing of the insert in pGEM3 was done with a modified T7 DNA polymerase (Sequenase) directly on the plasmid DNA using T7 or SP6 promoter sequence primers (Promega) according to the

30   manufacturer's protocol (USB, Cleveland, OH). The sequence was confirmed by repeatedly sequencing both strands of the insert.

**Example 4:**          **Results**

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15 positive plaques were selected after screening about $1 \times 10^6$ plaques from
λgt11 lactating breast cDNA library. The largest cDNA, BA46-1 was 1271 base pairs
long. A series of positive λgt11 clones were used to lysogenize Y1089 and the
resulting fusion protein containing induced cell extracts were analyzed by dot blot
5    analysis for reactivity with each of the monoclonal antibodies contained in the
screening cocktail.

It was found that monoclonal antibodies Mc8, Mc15 and Mc16 bound to all the
positive λgt11 lysogen extracts but not to the control λgt11 extract (not shown).
Monoclonal antibody Mc3, however, did not bind any of the lysates indicating that its
10   epitope requires either glycosylation, or secondary structure, or is not present in the
library.


**Example 5:**          **Partial RNA Sequence**

Single stranded RNA probes representing each strand of the BA46-1 cDNA
insert were prepared by subcloning into Gem3 and transcribing in vitro with T7 or SP6
15   polymerase.

Several neoplastic tumor cell lines were studied including 5 breast lines and a
lymphoid cell line of carcinomic origin for BA46-1 specific RNA. As shown in Figure
1 accompanying this patent, a single 2.2 kb RNA was detected in all cell lines tested.
This RNA is also detectable in the Raji cell line, but at a much lower level that requires
20   longer exposures and it, therefore, does not appear in Figure 1.

There was considerable variation in the observed expression levels of the 2.2
kb RNA that were detected in the different cell lines. The lung (A549), ovary (SKOV3)
and two breast (E11-G and HS578T) carcinoma cell lines accumulated from 10-50 fold
more transcript than the other cell lines.


25   **Example 6:**          **Specificity Studies**

Although the antibodies used to select the cDNA bound only  to breast
carcinomas by immunohistochemistry (Peterson et al, Hybridoma (1990), supra),
expression of the 2.2 kb RNA fragment that encodes the 46 Kdalton HMFG antigen is
expressed in many different cancer cell lines, such as carcinoma cell lines. The broad
30   specificity found for cancers from tissues of different origins, not only breast neoplastic
cells may be attributed to a deregulation of this gene in neoplastic tumors such as
carcinomas but not in normal tissue.

Normal epithelial tissue may also express the 46 Kdalton  app. MW HMFG
protein, but process it in a way that blocks the epitopes that are exposed in the breast

cell version of the protein by, for example, producing alterations in its glycosylation. The high molecular weight mucin-like protein of HMFG is also expressed in non-breast cancer cells such as carcinoma cells, but its altered processing in the pancreas, for example, leads to the exposure of different antigenic sites than in the breast (Lan,

5    M.S., Hollingworth, M.A., and Metzgar, T.S., Cancer Res. 50:2997 (1990)).

Example 7:                    Partial DNA Sequence

        The nucleotide and derived amino acid sequence of BA46-1 cDNA is shown in Table 1 below.

Table 1:        Partial DNA Sequence and Deduced Amino
                Acid Sequence of 46 Kdalton  HMFG antigen

```
           *      10      *      20      *      30      *      40
                  |              |              |              |
GAT TTC ATC CAT GAT GTT AAT AAA AAA CAC AAG GAG TTT GTG
Asp Phe Ile His Asp Val Asn Lys Lys His Lys Glu Phe Val


    *      50      *      60      *      70      *      80
           |              |              |              |
GGT AAC TGG AAC AAA AAC GCG GTG CAT GTC AAC CTG TTT GAG
Gly Asn Trp Asn Lys Asn Ala Val His Val Asn Leu Phe Glu


*      90      *      100     *      110     *      120     *
       |              |              |              |
ACC CCT GTG GAG GCT CAG TAC GTG AGA TTG TAC CCC ACG AGC
Thr Pro Val Glu Ala Gln Tyr Val Arg Leu Tyr Pro Thr Ser


    130     *      140     *      150     *      160     *
      |              |              |              |
TGC CAC ACG GCC TGC ACT CTG CGC TTT GAG CTA CTG GGC TGT
Cys His Yhr Ala Cys Thr Leu Arg Phe Glu Leu Leu Gly Cys


170     *      180     *      190     *      200     *      210
  |              |              |              |              |
GAG CTG AAC GGA TGC GCC AAT CCC CTG GGC CTG AAG AAT AAC
Glu Leu Asn Gly Cys Ala Asn Pro Leu Gly Leu Lys Asn Asn


       *      220     *      230     *      240     *      250
              |              |              |              |
AGC ATC CCT GAC AAG CAG ATC ACG GCC TCC AGC AGC TAC AAG
Ser Ile Pro Asp Lys Gln Ile Thr Ala Ser Ser Ser Tyr Lys


    *      260     *      270     *      280     *      290
           |              |              |              |
ACC TGG GGC TTG CAT CTC TTC AGC TGG AAC CCC TCC TAT GCA
Thr Trp Gly Leu His Leu Phe Ser Trp Asn Pro Ser Tyr Ala


*      300     *      310     *      320     *      330     *
       |              |              |              |
CGG CTG GAC AAG CAG GGC AAC TTC AAC GCC TGG GTT GCG GGG
Arg Leu Asp Lys Gln Gly Asn Phe Asn Ala Trp Val Ala Gly


    340     *      350     *      360     *      370     *
      |              |              |              |
AGC TAC GGT AAC GAT CAG TGG CTG CAG GTG GAC CTG GGC TCC
Ser Tyr Gly Asn Asp Gln Trp Leu Gln Val Asp Leu Gly Ser
```

**Table 1:**     **Partial DNA Sequence and Deduced Amino**
                            **Acid Sequence of 46 Kdalton HMFG Antigen (Cont'd.)**

```
   380       *     390       *    400       *    410       *    420
   |               |               |               |               |
  TCG AAG GAG GTG ACA GGC ATC ATC ACC CAG GGG GCC CGT AAC
  Ser Lys Glu Val Thr Gly Ile Ile Thr Gln Gly Ala Arg Asn

           *     430       *    440       *    450       *    460
                 |               |               |               |
  TTT GGC TCT GTC CAG TTT GTG GCA TCC TAC AAG GTT GCC TAC
  Phe Gly Ser Val Gln Phe Val Ala Ser Tyr Lys Val Ala Tyr

       *     470       *    480       *    490       *    500
             |               |               |               |
  AGT AAT GAC AGT GCG AAC TGG ACT GAG TAC CAG GAC CCC AGG
  Ser Asn Asp Ser Ala Asn Trp Thr Glu Tyr Gln Asp Pro Arg

   *     510       *    520       *    530       *    540       *
         |               |               |               |
  ACT GGC AGC AGT AAG ATC TTC CCT GGC AAC TGG GAC AAC CAC
  Thr Gly Ser Ser Lys Ile Phe Pro Gly Asn Trp Asp Asn His

       550       *    560       *    570       *    580       *
         |               |               |               |
  TCC CAC AAG AAG AAC TTG TTT GAG ACG CCC ATC CTG GCT CGC
  Ser His Lys Lys Asn Leu Phe Glu Thr Pro Ile Leu Ala Arg

   590       *    600       *    610       *    620       *    630
   |               |               |               |               |
  TAT GTG CGC ATC CTG CCT GTA GCC TGG CAC AAC CGC ATC GCC
  Tyr Val Arg Ile Leu Pro Val Ala Trp His Asn Arg Ile Ala

           *     640       *    650       *    660       *    670
                 |               |               |               |
  CTG CGC CTG GAG CTG CTG GGC TGT TAG TGG CCA CCT GCC ACC
  Leu Arg Leu Glu Leu Leu Gly Cys End    (■)

       *     680       *    690       *    700       *    710
             |               |               |               |
  CCC AGG TCT TCC TGC TTT CCA TGG GCC CGC TGC CTC TTG GCT

   *     720       *    730       *    740       *    750       *
         |               |               |               |
  TCT CAG CCC CTT TAA ATC ACC ATA GGG CTG GGG ACT GGG GAA

       760       *    770       *    780       *    790       *
         |               |               |               |
  GGG GAG GGT GTT CAG AGG CAG CAC CAC CAC ACA GTC ACC CCT

   800       *    810       *    820       *    830       *    840
   |               |               |               |               |
  CCC TCC CTC TTT CCC ACC CTC CAC CTC TCA CGG GCC CTG CCC

           *     850       *    860       *    870       *    880
                 |               |               |               |
  CAG CCC CTA AGC CCC GTC CCC TAA CCC CCA GTC CTC ACT GTC

       *     890       *    900       *    910       *    920
             |               |               |               |
  CTG TTT TCT TAG GCA CTG AGG GAT CTG AGT AGG TCT GGG ATG

   *     930       *    940       *    950       *    960       *
         |               |               |               |
  GAC AGG AAA GGG CAA AGT AGG GCG TGT GGT TTC CCT GCC CCT
```

**Table 1:**      **Partial DNA Sequence and Deduced Amino
Acid Sequence of 46 Kdalton    HMFG Antigen (Cont'd.)**

```
    970      *      980       *    990         *     1000        *
     |              |              |                 |
GTC CGG ACC GCC GAT CCC AGG TGC GTG TGT CTC TGT CTC TCC

  1010    *  1020       *    1030       *    1040      *   1050
   |          |              |              |              |
TAG CCC CTC TCT CAC ACA TCA CAT TCC CAT GGT GGC CTC AAG

       *   1060       *   1070       *   1080       *   1090
                       |              |                 |
AAA GGC CCG GAA GCC CCA GGC TGG AGA TAA CAG CCT CTT GCC

      *    1100       *  1110       *    1120       *   1130
           |              |              |              |
CGT CGG CCC TGC GTC GGC CCT GGG GTA CCA TGT GCC ACA ACT

  *  1140       *   1150       *   1160       *   1170        *
      |              |              |              |
GCT GTG GCC CCC TGT CCC CAA GAC ACT TCC CCT TGT CTC CCT

  1180       *   1190       *   1200       *   1210        *
   |              |              |              |
GGT TGC CTC TCT TGC CCC TTG TCC TGA AGC CCA GCG ACA CAG

  1220    *  1230       *   1240       *   1250       *   1260
   |          |              |              |              |
AAG GGG GTG GGG CGG GTC TAT GGG GAG AAA GGG AGC GAG GTC

       *   1270       *   1280       *   1290       *   1300
            |              |              |              |
AGA GGA GGG CAT GGG TTG GCA GGG TGG GCG TTT GGG GCC CTC

      *   1310       *   1320       *   1330       *   1340
           |              |              |              |
ATG CTG GCT TTT CAC CCC AGA GGA CAC AGG CAG CTT CCA AAA

  *  1350       *   1360       *   1370       *   1380
      |              |              |              |
TAT ATT TAT CTT CTT CAC GGG AAA AAA AAA AAA AAA ACC G  (□)
```

(□) : SEQ. ID. No. 1
(■) : SEQ. ID. No. 2
Potential n-linked glycosylation sites are underlined

The partial ORF sequence is 217 amino acids long and compounds to a theoretical molecular weight of about 24 Kdalton , representing the C-terminus of the complete protein. There are four potential sites for n-linked glycosylation and the
5    polypeptide sequence is asparagine and leucine rich.

**Example 8:**       **Homology to Clotting Factors**

A comparison of the nucleotide sequence to the EMBL database using FSTNSCAN (PCGENE) revealed extended homology with human serum factors V and
10   VIII and protein C. The partial deduced protein sequence, however, shares identity only with factors V and VIII but not with protein C since the homology at the nucleotide

level is found in an intervening sequence (See, Table 2  below).


**Table 2:**     **Comparison of Deduced BA46-1 Amino Acid Sequence**
                **with C-terminal Human Serum Factors V and VIII**

```
46 Kdalton      F I H D V N K K H K E F V G N W N K N A V H V N
FAV             F K G N S T R N V M Y F N G N S D A S T I K E N
FAVIII          Y R G N S T G T L M V F F G N V D S S G I K H N

        L F E T P V E A Q Y V R L Y P T S C H T A C T L R F E L
        Q F D P P I V A R Y I R I S P T R A Y N R P T L R L E L
        I F N P P I I A R Y I R L H P T H Y S I R S T L R M E L

        L G C E L N G C A N P L G L K N N S I P D K Q I T A S S
        Q G C E V N G C S T P L G M E N G K I E N K Q I T A S S
        M G C D L N S C S M P L G M E S K A I S D A Q I T A S S

        S Y K T W G L H L F S W N P S Y A R L D K Q G N F N A W
        F K K S W W G D Y - - W E P F R A R L N A Q G R V N A W
        Y F T N M F A T - - - W S P S K A R L H L Q G R S N A W

        V A G S Y G N D Q W L Q V D L G S S K E V T G I I T Q G
        Q A K A N N N K Q W L E I D L L K I K K I T A I I T Q G
        R P Q V N N P K E W L Q V D F Q K T M K V T G V T T Q G

        A R N F G S V Q F V A S Y K V A Y S N D S A N W T E Y Q
        C K S L S S E M Y V K S Y T I H Y S E Q G V E W K P Y R
        V K S L L T E M Y V K E F L I S S S Q D G H Q W T L F F

        D P R T G S S K I F P G N W D N H S H K K N L F E T P I
        L K S S M V D K I F E G N T N T K G H V K N F F N P P I
        Q N - - G K V K V F Q G N Q D S F T P V V N S L D P P L

        L A R Y V R I L P V A W H N R I A L R L E L L G C
        I S R F I R V I P K T W N Q S I A L R L E L F G C D - -
        L T R Y L R I H P Q S W V H Q I A L R M E V L G C E A Q

              (SEQ. ID. No. 3)
        - I Y (SEQ. ID. No. 4)
        D L Y (SEQ. ID. No. 5)
```

An  arrow  indicates  function  of  C  and  C2  repeats

----

There is about 43% identity of BA46 to Factor V and about 38% to factor VIII. The region of factors V and VIII in Table 2 share about 47% identity.


**Example 9:**              **Amino Acid Sequence**

The analysis of the derived amino acid sequence of the 46 Kdalton  app. MW
5  protein is consistent with its description as a glycosylated protein containing four N-linked glycosylation sites.  Since the 46 Kdalton  app. MW protein has homology to both factors V and VIII, there may be a common ancestral protein to these serum clotting factors.  The homology is in the C1C2 region of the light chain of factor VIII (Arai, M., Scandella, D., and Hoyer, L.W.,  J. Clin. Invest. 83:1978-1984 (1989)).

Arai et al have shown that human antibodies that bind the C1C2 region of the light chain from hemophiliacs treated with factor VIII inhibit factor VIII by preventing the interaction of factor VIII with phospholipids and that it is implicated in phospholipid binding. It is likely that the similar sequence is also important for phospholipid binding

5   in the 46 Kdalton glycoprotein.

The C-terminal portion could serve as a novel "anchor" sequence for the 46 Kdalton app. MW protein or it could be involved in the binding of the mucin/membrane to the phospholipids on the surface of the growing milk fat droplet (Long, C.A., and Patton, S., J. Dairy Sci. 61:1392-1399 (1978)). It could also be involved in the

10  assembly of the mucin complex at the plasma membrane surface.

### Example 10:         Screening of cDNA Libraries

The ZR75 λgt11 cDNA library was screened using an isolated cloned LB21 sequence encompassing bases 562 through 1838 of the total 1934 base pairs of the 46 Kdalton app. MW BA46 clone and labeled with $P^{32}$ using random primers. The

15  cDNA clone LB21 utilized herein comprises a portion of the C-terminal region of the BA46 cDNA, and its cloning and expression in E. coli as an expression vector (pEX/LB21) have been described by Larocca, et al (Larocca et al, Molecular Cloning and Expression of Breast Mucin-associated Antigens", in Breast Epithelial Antigens: Molecular Biology to Clinical Applications, Ceriani R.L. Ed., PP. 35-44, New York

20  Plenum Press (1991)). The bacteriophages were plated at a density of 30,000 pfu / 150 mm plates with E. coli Y1090, and blotted with nitrocellulose filters as described by Larocca et al. (Larocca et al, "Cloning and sequencing of a complementary DNA encoding a Mr 70, 000 human breast epithelial mucin-associated antigen", Cancer Res. 50:5925-5930 (1990)). The $P^{32}$-labeled LB21 clones were then screened, and

25  positive plaques visualized by autoradiography, picked and plaque purified. The inserts were amplified by PCR using forward and reverse primers for the adjacent λgt11 bacteriophage sequences, subcloned into pGEM3 (Promega, Madison, WI) at the EcoR1 site, and sequenced by the Sanger method of dideoxynucleotide chain termination as described by Larocca et al, "Cloning and sequencing of a complementary DNA

30  encoding a Mr 70, 000 human breast epithelial mucin-associated antigen", (Cancer Res.50:5925-5930 (1990); Sanger et al, "DNA sequencing with chain-terminating inhibitors", PNAS (USA) 74:463-5467 (1977)). The screening of the human breast λgt11 cDNA library was done by PCR using an up-stream primer for the λgt11 vector and several downstream primers for known sequences in the 5' region.

<u>Example 11</u>:          **PCR Primer Synthesis**

The primers listed in Table 3 below were synthesized for the PCR using an Applied Biosystems model 391 PCR-MATE DNA synthesizer by the phosphoramidite method. The oligonucleotides were run on a 8M urea/10% polyacrylamide denaturing

5  gel to assess their purity and integrity.

<u>Table 3</u>: **DNA Sequence of Primers Utilized**

| Primer | Type[+] | DNA Sequence |
|--------|---------|--------------|
| 5'-46KRT[*] | Antisense | 5'-GGTGTCCAGGCATTGACCAT-3' |
| BA46 P-2[□] | Antisense | 5'-GCTGCAAACCCAAGAAGGTCAC-3' |
| BA46 P-A[†] | Antisense | 5'-TAAGGCACGTGCAGGTGTACGA-3' |
| BA46P-C | Antisense | 5'-TTGGAACAGATATCCAGGGCGA-3' |
| λgt11 Fwd. | Sense | 5'-GGTGGCGACGACTCCTGGAGCCCG-3' |
| λgt11 Rev. | Sense | 5'-TTGACACCAGACCAACTGGTAATG-3' |

[*]  Sense or antisense indicates the sequence of the primer is either identical or complementary to    the BA46 mRNA, respectively.
[▸]  Bases 337-356 of the $M_r$ 46,000 gene coding sequence.
[□]  Bases 277-298 of the $M_r$ 46,000 gene coding sequence.
[·]  Bases 154-175 of the $M_r$ 46,000 gene coding sequence.
    Bases 65-86  of the $M_r$ 46,000 gene coding sequence.

<u>Example 12</u>:          **PCR Conditions**

The PCR was carried out in a 50 ml reaction volume using the GeneAmp PCR kit (Perkin Elmer Cetus, CT). The samples were run under "hot start" conditions, in 0.2 ml PCR MicroAmp™ tubes using the GeneAmp™ PCR system 9600 (Perkin Elmer-

5  Cetus, CT). The following conditions were used for PCR screening of the human breast cDNA library. In each tube, 5 $\mu$l of  a 1:10 dilution of the cDNA library (equivalent to approximately $0.65 \times 10^6$ independent cell clones) were added to 40 $\mu$l PCR master reaction mixture containing 5 $\mu$l of 10 x PCR buffer II (100  mM Tris-HCl, pH 8.3, 500 mM KCl), 4 $\mu$l of 25 mM $MgCl_2$, 1 $\mu$l each of 10 mM dNTP,  17 $\mu$l sterile

10  water, 5 $\mu$l of  2 mM ($M_o$=46,000) gene specific antisense primer, and 5 $\mu$l of 2 mM λgt11 sense primer. The samples were heated to 95°C for 2 min in the PCR system 9600, allowed to cool to 75°C and held at this temperature while 1.25 units of "AmpliTaq" DNA polymerase were added in 5 $\mu$l of 1 x Taq buffer. Primer annealing was initially performed at 62°C for 30 sec followed by 35 cycles of denaturation at

95°C, ramping to 64°C in 30 sec and annealing and extending at 64°C for 30 sec. After completion of the PCR cycles, the reaction mixtures were extended at 72°C for 7 min and cooled to 4°C.

5   The amplified DNA was subjected to gel electrophoresis, ethidium bromide stained, and visualized under UV light. A smear of DNA bands relating to incomplete 5'-ends of the BA46 cDNA was cloned directly into pCR™ II using a TA cloning kit from Invitrogen (San Diego, CA). This method took advantage of the non-template dependent activity of Taq polymerase that adds a single dA to the 3' end of PCR duplex products (Marchuck et al, "Nucleic Acids Res. 19:1154 (1991)). Single 3'dT

10   overhangs in the vector, pCR™ II, allowed for PCR product insertion.


**Example 13:**          **Nucleotide sequencing**

The cDNA clones isolated from the screening of the ZR75 breast cell λgt11 cDNA library were subcloned into a pGEM3 plasmid and sequenced by the

15   didioxynucleotide chain termination method as described by Larocca et al. (Larocca, et al, "Cloning and sequencing of a complementary DNA encoding a M, 70,000 human breast epithelial mucin-associated antigen", Cancer Res. 50:5925 (1990)). Positive clones obtained from the human breast library by the PCR method were picked, amplified and phage DNA isolated by the method of lysis by boiling as described by

20   Sambrook et al. (Sambrook et al, in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, Cold Spring Harbor Press (1989)). EcoR1 digested inserts were screened by gel electrophoresis choosing DNA fragments of the size expected. The size of the chosen DNA fragments was assessed from the fact that a single 2.2 kilobase RNA was detected in carcinoma cell lines, by Northern blotting as described

25   above (Larocca et al., "A 46 Kdalton human milk fat globule glycoprotein that is highly expressed in carcinoma cells has homology with human clotting factors V and VIII", Cancer Res. 51:4994 (1991)).

High yields of pure supercoiled plasmid DNA for sequencing were prepared from overnight cultures of selected clones using the Qiagen plasmid minipreparation kit

30   (Qiagen Inc., CA). The insert in pCR™ II was sequenced by the method of dideoxynucleotide chain termination (Sanger, supra), using a modified T7 DNA polymerase (Sequenase Version 2.0) under the conditions recommended by the supplier (USB Corp., Cleveland, OH). Both strands were sequenced by priming in the plasmid with either M13 reverse or M13 (-40) forward primers (USB Corp., OH). The analysis

35   of the sequence was performed using GeneWorks software (IntelliGenetics, Inc., Mountain View, CA).

The breast cDNA library was screened by PCR, priming with the λgt11 Fwd. and the BA46 P-2 primers described above. The latter primer comprises bases 277-298, 37 bases within the 5' end of the sequence obtained by screening the ZR75 library and provided an extra 197 bases of the 5' end of the M,=46,000 cDNA. A second BA46

5    gene specific primer (BA46 P-A, bases 154-175) was synthesized within the extended 5' end sequence and used to further screen the breast cDNA library. The BA46 cDNA was further extended by 43 bases including the start codon.

### Example 14:          Confirmation of 5' end of BA 46 cDNA

The 5'-end of the 46 Kdalton  BA46 cDNA was confirmed by screening mRNA

10   from a breast cell line using the 5'-AmpliFinder RACE kit (Clonetech, Palo Alto). PolyA$^+$ RNA was prepared from the "ELL-G" breast cell line using a "FastTrack mRNA isolation kit, version 3.2" (Invitrogen, San Diego, CA). cDNA was synthesized from 2µg of the ELL-G mRNA using the antisense gene specific primer "5'-46KRT" (bases 337-356) with the 5'-AmpliFinder RACE kit. The RNA template was then hydrolyzed

15   with NaOH, and the cDNA purified by binding to a glass matrix support (GENO-BIND™ particles) in preparation for the ligation of a single stranded anchor oligonucleotide to the 3' end of the cDNA.   The gene was then amplified by PCR using the nested antisense gene-specific primers "BA46 P-A and BA46 P-C" and a primer complementary to the anchor. PCR was carried out as described above under "hot

20   start" conditions using AmpliTaq DNA polymerase, Stoffel fragment (Perkin Elmer-Cetus). Each 50 µl of PCR reaction mixture contained a 1:100 dilution of the anchor ligation mix, 1 x Stoffel buffer (10 mM KCl, 10 mM Tris HCl, pH 8.3), 3 mM MgCl$_2$, 0.2 mM each of dNTP and 0.3 µM of sense and antisense (both) primers.

The samples were heated to 98°C for 1 min, cooled to 75°C, and  5 units of the

25   Stoffel fragment added. The primers were  initially annealed and extended at 64°C for 30 seconds. Two PCR methods were used to amplify the gene. The first consisted of 10 cycles of denaturing at 97°C for 10 seconds and annealing and extending at 62°C for 25 seconds. This was followed by 30 cycles of denaturing at 95°C for 10 seconds and annealing and extending at 60°C for 25 seconds. A final extension at 72°C for 7

30   min ensured that all the templates were completed. The PCR products were visualized on a 2% agarose gel, and then cloned into the pCR II vector (TA cloning kit, Invitrogen) for dideoxynucleotide sequencing.

<u>Example 15</u>:              **Complete DNA Sequence of Polynucleotide**
                              **Encoding 46 Kdalton HMFG Antigen**

The partial cDNA sequence for the 46 Kdalton HMFG antigen clone (BA46) provided in Example 7 above was completed. A complete DNA sequence was obtained

5   by screening and PCR amplification of a cDNA library, and the rapid amplification of cDNA ends (RACE) method. The breast carcinoma cell line ZR75 cDNA library was screened with the partial cDNA clone LB21 of the BA46 sequence shown above, and labeled with $P^{32}$.

Two new cDNA clones were isolated, which provided further information on the

10  cDNA segment encoding the 46 Kdalton HMFG antigen. These two clones completed the 3' end (97 bases to the polyadenylation site), and extended by 267 bases the 5' end of the cDNA sequence.

The sequence of the 5' end of the cDNA was completed by screening a human breast λgt11 cDNA by PCR amplification using antisense primers on the 5' end of the

15  BA46 cDNA, and sense primers within the λgt11 cloning vector. The latter PCR method allowed the complete sequencing of the open reading frame (ORF) of the 46 Kdalton HMFG polypeptide (BA46) cDNA. The ORF sequence was confirmed and the non-coding 5' end was sequenced by the RACE method. Each cDNA insert was sequenced in both directions on at least two independent isolates to verify the

20  sequence.

The entire cDNA contains 1934 bases and an ORF 1161 nucleotides encoding 387 nucleotides as shown in Table 4 below.

<u>Table 4</u>:           **Complete DNA Sequence Encoding the 46 Kdalton**
                        **HMFG Antigen & Deduced Amino Acid Sequence**

```
  1  AGAACCCCGCGGGGTCTGAGCAGCCCAGCGTGCCCATTCCAGCGCCCGCGTCCCCGCAGC

 61  ATGCCGCGCCCCCGCCTGCTGGCCGCGCTGTGCGGCGCGCTGCTCTGCGCCCCCAGCCTC
  1   M   P   R   P   R   L   L   A   A   L   C   G   A   L   L   C   A   P   S   L

121  CTCGTCGCCCTGGATATCTGTTCCAAAAACCCCTGCCACAACGGTGGTTTATGCGAGGAG
 21   L   V   A   L   D   I   C   S   K   N   P   C   H   N   G   G   L   C   E   E

181  ATTTCCCAAGAAGTGCGAGGAGATGTCTTCCCCTCGTACACCTGCACGTGCCTTAAGGGC
 41   I   S   Q   E   V   R   G   D   V   F   P   S   Y   T   C   T   C   L   K   G

241  TACGCGGGCAACCACTGTGAGACGAAATGTGTCGAGCCACTGGGCATGGAGAATGGGAAC
 61   Y   A   G   N   H   C   E   T   K   C   V   E   P   L   G   M   E   N   G   N

301  ATTGCCAACTCACAGATCGCCGCCTCATCTGTGCGTGTGACCTTCTTGGGTTTGCAGCAT
 81   I   A   N   S   Q   I   A   A   S   S   V   R   V   T   F   L   G   L   Q   H

361  TGGGTCCCGGAGCTGGCCCGCCTGAACCGCGCAGGCATGGTCAATGCCTGGACACCCAGC
101   W   V   P   E   L   A   R   L   N   R   A   G   M   V   N   A   W   T   P   S
```

46

**Table 4:**   Complete DNA Sequence Encoding the 46 Kdalton
               HMFG Antigen & Deduced Amino Acid Sequence (Cont'd)

```
 421 AGCAATGACGATAACCCCTGGATCCAGGTGAACCTGCTGCGGAGGATGTGGGTAACAGGT
 121  S   N   D   D   N   P   W   I   Q   V   N   L   L   R   R   M   W   V   T   G

 481 GTGGTGACGCAGGGTGCCAGCCGCTTGGCCAGTCATGAGTACCTGAAGGCCTTCAAGGTG
 141  V   V   T   Q   G   A   S   R   L   A   S   H   E   Y   L   K   A   F   K   V

 541 GCCTACAGCCTTAATGGACACGAATTCGATTTCATCCATGATGTTAATAAAAAACACAAG
 161  A   Y   S   L   N   G   H   E   F   D   F   I   H   D   V   N   K   K   H   K

 601 GAGTTTGTGGGTAACTGGAACAAAAACGCGGTGCATGTCAACCTGTTTGAGACCCCTGTG
 181  E   F   V   G   N   W   N   K   N   A   V   H   V   N   L   F   E   T   P   V

 661 GAGGCTCAGTACGTGAGATTGTACCCCACGAGCTGCCACACGGCCTGCACTCTGCGCTTT
 201  E   A   Q   Y   V   R   L   Y   P   T   S   C   H   T   A   C   T   L   R   F

 721 GAGCTACTGGGCTGTGAGCTGAACGGATGCGCCAATCCCCTGGGCCTGAAGAATAACAGC
 221  E   L   L   G   C   E   L   N   G   C   A   N   P   L   G   L   K   N   N   S

 781 ATCCCTGACAAGCAGATCACGGCCTCCAGCAGCTACAAGACCTGGGGCTTGCATCTCTTC
 241  I   P   D   K   Q   I   T   A   S   S   S   Y   K   T   W   G   L   H   L   F

 841 AGCTGGAACCCCTCCTATGCACGGCTGGACAAGCAGGGCAACTTCAACGCCTGGGTTGCG
 261  S   W   N   P   S   Y   A   R   L   D   K   Q   G   N   F   N   A   W   V   A

 901 GGGAGCTACGGTAACGATCAGTGGCTGCAGGTGGACCTGGGCTCCTCGAAGGAGGTGACA
 281  G   S   Y   G   N   D   Q   W   L   Q   V   D   L   G   S   S   K   E   V   T

 961 GGCATCATCACCCAGGGGGCCCGTAACTTTGGCTCTGTCCAGTTTGTGGCATCCTACAAG
 301  G   I   I   T   Q   G   A   R   N   F   G   S   V   Q   F   V   A   S   Y   K

1021 GTTGCCTACAGTAATGACAGTGCGAACTGGACTGAGTACCAGGACCCCAGGACTGGCAGC
 321  V   A   Y   S   N   D   S   A   N   W   T   E   Y   Q   D   P   R   T   G   S

1081 AGTAAGATCTTCCCTGGCAACTGGGACAACCACTCCCACAAGAAGAACTTGTTTGAGACG
 341  S   K   I   F   P   G   N   W   D   N   H   S   H   K   K   N   L   F   E   T

1141 CCCATCCTGGCTCGCTATGTGCGCATCCTGCCTGTAGCCTGGCACAACCGCATCGCCCTG
 361  P   I   L   A   R   Y   V   R   I   L   P   V   A   W   H   N   R   I   A   L

1201 CGCCTGGAGCTGCTGGGCTGTTAGTGGCCACCTGCCACCCCCAGGTCTTCCTGCTTTCCA
 381  R   L   E   L   L   G   C                         (SEQ. ID No: 6)

1261 TGGGCCCGCTGCCTCTTGGCTTCTCAGCCCCTTTAAATCACCATAGGGCTGGGGACTGGG
1321 GAAGGGGAGGGTGTTCAGAGGCAGCACCACCACACAGTCACCCCTCCCTCCCTCTTTCCC
1381 ACCCTCCACCTCTCACGGGCCCTGCCCCAGCCCCTAAGCCCCGTCCCCTAACCCCCAGTC
1441 CTCACTGTCCTGTTTTCTTAGGCACTGAGGGATCTGAGTAGGTCTGGGATGGACAGGAAA
1501 GGGCAAAGTAGGGCGTGTGGTTTCCCTGCCCCTGTCCGGACCGCCGATCCCAGGTGCGTG
1561 TGTCTCTGTCTCTCCTAGCCCCTCTCTCACACATCACATTCCCATGGTGGCCTCAAGAAA
1621 GGCCCGGAAGCCCCAGGCTGGAGATAACAGCCTCTTGCCCGTCGGCCCTGCGTCGGCCCT
1681 GGGGTACCATGTGCCACAACTGCTGTGGCCCCCTGTCCCAAGACACTTCCCCTTGTCTC
1741 CCTGGTTGCCTCTCTTGCCCCTTGTCCTGAAGCCCAGCGACACAGAAGGGGGTGGGGCGG
1801 GTCTATGGGGAGAAAGGGAGCGAGGTCAGAGGAGCCGGCATGGGTTGGCAGGGTGGGCGT
1861 TTGGGGCCCTCATGCTGGCTTTTCACCCCAGAGGACACAGGCAGCTTCCAAAATATATTT
1921 ATCTTCTTCACGGG                                 (SEQ. ID No: 7)
```

47

**Example 16:**      **Characteristics of BA46 DNA Segment**

The cDNA sequence is characterized by a 3' poly(A) tail and an untranslated 3' region of 713 nucleotides. The usual consensus polyadenylation signal sequence of AATAAA is not found but the sequence AATATA is found in the same position relative

5    to the AATACA sequence found in the mouse MFGE8 cDNA sequence, 17 nucleotides upstream of the poly(A) tail (Stubbs et al., "cDNA cloning of a mouse mammary epithelial cell surface protein reveals the existence of epidermal growth factor-like domains linked to factor VIII-like sequences", PNAS (USA) 87:8417-8421 (1990)). The AATACA and AATATA sequences are considered alternate polyadenylation

10   signals. At the 5' end of the cDNA, the first ATG start codon is preceded by the sequence GCAGC, which is frequently associated with AGT start codons. The non-coding 5' region contains 60 nucleotides.

**Example 17:**      **Homology of 46 Kdalton Polypeptide and**
                     **Murine Milk Fat Globule Antigen MFGE8**

15   The BA46 cDNA sequence has considerable homology with the cDNA of a mouse milk fat globule glycoprotein MFGE8 of 66/55 Kdalton described by Stubbs et al, supra. The nucleotide sequence of the BA46 open reading frame has 76% identity with that of MFGE8. The 5' and 3' non-coding regions have 71% and 62% identities, respectively. The greatest % identity is present in the nucleotide sequences encoding

20   the function domains, within the open reading frame, that are shared by the two encoded proteins as shown in Table 5 below.

**Table 5:**      **Homologies between 46 Kdalton HMFG Antigen**
                  **Polypeptide and MMFG Antigen Polypeptide (MFGE8)**

```
46KORF    - MPRPRLLAALCGALLCAPSLLVALD----------------------------- -25
            :   :.::::::: :::: :  :
MFGE8PRO  - MQVSRVLAALCGMLLCASGLFAASGDFCDSSLCLNGGTCLTGQDNDIYCLCPBGF-like -55

46KORF    - -----------ICSKNPCHNGGLCBBISQBVRGDVFPSYTCTCLKGYAGNHCBT- -68
            ::: ::: :   :        ::::.: : : : ::.: ::::
MFGE8PRO  - TGLVCNBTBRGPCSPNPCYNDAKCLVTLDTQRGDIFTBYICQCPVGYSGIHCBTB -110

46KORF    - --------------------------------KCVBPLGMBNGNIANSQIA -87
                                              .:  :::: : :: :::.
MFGE8PRO  - TNYYNLDGBYMFTTAVPNTAVPTPAPTPDLSNNLASRCSTQLGMBGGAIADSQIS -165

46KORF    - ASSVRVTFLGLQHWVPBLARLNRAGMVNAWTPSSNDDNPWIQVNLLRRMWVTGVV -142
            :: : . :.:::  :  :::::: :.:.:::  :   :  ::::::::::.: :.::.
MFGE8PRO  - ASYVYMGPMGLQRWGPBLARLYRTGIVNAWHASNYDSLPWIQVNLLRKMRVSGVM -220

46KORF    - TQGASRLASHBYLKAFKVAYSLNGHBPDPIHDVNKKHKBFVGNWNKNAVHVNLFB -197
            ::::::      :::::.:::::::: :  :.:: :    :::.::  :..  ::.:
MFGE8PRO  - TQGASRAGRABYLKTFKVAYSLDGRKFBFIQDBSGGDKBFLGNLDNNSLKVNMFN -275
```

Table 5:        Homologies between 46 Kdalton  HMFG Antigen Polypeptide
                and MMFG Antigen Polypeptide (MFGE8) (Cont'd)

```
46KORF    - TPVEAQYVRLYPTSCHTACTLRFELLGCELNGCANPLGLKNNSIPDKQITASSSY -252
            .:: :.:::: ::: :::::::::::: :: :::::::.::: :..:::::
MFGE8PRO  - PTLEAEYIRLYPVSCHRGCTLRFELLGCELHGCLEPLGLKNNTIPDSQMSASSSY -330

46KORF    - KTWGLHLFSWNPSYARLDKQGNFNAWVAGSYGNDQWLQVDLGSSKEVTGIITQGA -307
            ::: : : : : ::: :: ::: : : :::::::. . ::::::::
MFGE8PRO  - KTWNLRAFGWYPHLGRLDNQGKINAWTAQSNSAKEWLQVDLGTQRQVTGIITQGA -385

46KORF    - RNPGSVQFVASYKVAYSNDSANWTEYQDPRTGSSKIPPGNWDNHSHKKNLFETPI -362
            : :: .:.: ::::: : : .:: : . ::::.: :: :: ::::::: :
MFGE8PRO  - RDFGHIQYVESYKVAHSDDGVQWTVYEEQ--GSSKVPQGNLDNNSHKKNIPEKPF -438

46KORF    - LARYVRILPVAWHNRIALRLELLGC -387
            .:: ::.::::.:::::.:::::::
MFGE8PRO  - MARKVRVLPVSWHNRITLRLELLGC -463
```

* (SEQ. ID No: 8)
+ (SEQ. ID No: 9)
. Similar Amino Acid
: Identical Amino Acid

## Example 18:        Epitope mapping

Overlapping peptide hexamers spanning amino acids 330-382 of the 46 Kdalton
HMFG polypeptide (BA46) sequence were synthesized onto the ends of polyethylene
pins using an Epitope Scanning Kit (Cambridge Research Biochemicals, Cambridge, UK)

5   as described by Geysen et al. (Geysen, et al. "Use of a peptide synthesis to probe vital
antigens for epitopes to a resolution of a single amino acid", PNAS (USA) 81:3998-
4002 (1984)). The polyethylene pins are arranged in an 8 x 12 configuration that fit
into a 96 well microliter dish and were supplied with a $\beta$-alanine attached to the ends
to which the amino acids are added, consecutively using pentafluorophenyl active

10  esters of fluorenylmethyloxycarbonyl (Fmoc)-L-amino acids.   Each consecutive
overlapping hexamer or octamer differed from the previous one by a single amino acid
and were synthesized to span amino acids 330-382 of the BA46 peptide so that every
combination of hexamer or octamer was present.   The binding of monoclonal
antibodies Mc3, Mc8, Mc15, Mc16 raised against the BA46 antigen as described by

15  Peterson et al (Peterson et al., "Biochemical and histological characterization of
antigens preferentially expressed on the surface and cytoplasm of breast carcinoma
cells identified by monoclonal antibodies against the human milk fat globule",
Hybridoma 9:221-235 (1990)) to the synthetic peptides was tested using the ELISA
method with horse radish peroxidase-conjugated goat anti-mouse IgG (Promega,

20  Madison, WI), and color development with 2,2' azino bis (3-ethyl benzothiazdine-6-

sulfuric acid (Sigma, St. Louis, MO).

### Example 19:          Open Reading Frame and Antibody
####                      Binding Characteristics

The largest open reading frame of the BA46 cDNA encodes a protein of 387

5  amino acids with an estimated molecular weight of 43,123 Kdalton s.  The actual

correspondence of the cDNA cloned to the BA46 glycoprotein antigen isolated from the

HMFG was shown by the correlation of the BA46 mRNA with the expression to the

BA46 antigen in different breast cell lines (Larocca et al., "A 46 Kdalton human milk

fat globule glycoprotein that is highly expressed in carcinoma cells has homology with

10  human clotting factors V and VIII", Cancer Res. 51: 994 (1991)) and the binding of

the monoclonal antibodies used to in the cDNA screening to the pEX/LB21 fusion

protein expressed in E. coli (Larocca et al., "Molecular cloning and expression of breast

mucin-associated antigens", in Breast Epithelial Antigens: Molecular Biology to Clinical

Applications,  R.L. Ceriani, Ed., pp. 35-44, New York Plenum Press (1991)).  In

15  addition, 5 defined and distinct amino acid sequence epitopes in the C-terminal end of

the protein were determined by epitope mapping for two monoclonal antibodies of the

cocktail used in the original screening of the cDNA library (Mc8 = DPRTG; and Mc16

= SSKIF) (See, Table 4 above).  The two other monoclonal antibodies (Mc3, Mc15)

neither bind to the pEX/LB21 fusion protein nor to any of the peptide hexamers used

20  in the epitope mapping of the C-terminal region (amino acids 330 - 382) of the 46

Kdalton polypeptide.

### Example 20:          Homology of 46 Kdalton  Polypeptide
####                      (BA46) with Other Known Proteins

The amino acid sequence deduced with the help of the PC/GENE DNA and the

25  protein analysis program (IntelliGenetics, Inc.) revealed the existence of  homologies

with several functional domains.  At the N-terminal end, there is a hydrophobic region

after the Met start codon which most likely corresponds to a signal peptide.  Cleavage

most likely occurs between the $val_{21}$ and $ala_{22}$, leaving a cleaved peptide of 21 amino

acids plus the methionine.  This cleavage results in a processed polypeptide of 40,862

30  Kdalton s.  Amino acids 46 to 48 represent a known cell adhesion sequence (RGD),

and following this is an EGF-like domain (amino acids 55 to 66).  The C-terminal end,

starting at amino acid 69 comprises a domain with homology to the C/C2 region of

human coagulation factors V and VIII, a portion of which is shown in Table 1 above.

The sequence contains four potential N-linked glycosylation sites, all present in the

35  C1C2-like domain, numerous potential O-linked glycosylation sites, disulfide linkages,

50

and phosphorylation sites (protein kinase C and casein kinase II). The greatest homology to other proteins is seen with the 66/55 Kdalton antigen MFGE8 isolated from mouse milk fat globule (Stubbs et al., supra) as shown in Table 5 above and Table 6 below.

**Table 6**:      **Comparison of Deduced 46 Kdalton H M F G Antigen Sequence to other Protein Sequences**

```
BA46C1   KCVEPLGMENGNIANSQIAASSVRVT--PLGLQHWVPELARLNRAGMVNA
MFGE8C1  RCSTQLGMBGGAIADSQISASYVYMG--PMGLQRWGPELARLYRTGIVNA
BA46C2   GCANPLGLKNNSIPDKQITASSSYKTWGLHLFS-WNPSYARLDKQGNPNA
MFGE8C2  GCLEPLGLKNNTIPDSQMSASSSYKTWNLRAFG-WYPHLGRLDNQGKINA
FA5C2    GCSTPLGMBNGKIENKQITASSPKKSW-WGDY--WBPPRARLNAQGRVNA
FA8C2    SCSMPLGMESKAISDAQITASSYPTN----MPATWSPSKARLHLQGRSNA
FA5C1    DCRMPMGLSTGIISDSQIKASBP--------LGYWBPRLARLNNGGSYNA
FA8C1    KCQTPLGMASGHIRDFQITASGQ--------YGQWAPKLARLHYSGSINA
A5C1     QCKBALGMESGBIHPDQISVSSQYSM-------NWSABRSRLNY--VBNG
A5C2     PCSRMLGMVSGLISDSQITASSQVDR-------NWVPBLARLVT--SRSG
DDRC1    KCRYALGMQDRTIPDSDISASS---SWS----DSTAARHSRLESSDGDGA
DISC                                              DGSBA
GP55     GCLEPL                         WGPBLAR
BAND16                                 WAPBLAR
```

```
BA46C1   WT----PSSNDDNPWIQVNLLRRMWVTGVVTQGA--SRLASHBYLKAFKV
MFGE8C1  WH----ASNYDSLPWIQVNLLRKMRVSGVMTQGA--SRAGRABYLKTFKV
BA46C2   WV----AGSYGNDQWLQVDLGSSKBVTGIITQGA--RNFGSVQFVASYKV
MFGE8C2  WT----AQSNSAKBWLQVDLGTQRQVTGIITQGA--RDFGHIQYVBSYKV
FA5C2    WQ----AKANNNKQWLBIDLLKIKKITAIITQGC--KSLSSBMYVKSYTI
FA8C2    WR----PQVNNPKBWLQVDPQKTMKVTGVTTQGV--KSLLTSMYVKBFLI
FA5C1    WSVEKLAABFASKPWIQVDMQKEVIITGIQTQGA--KHYLKSCYTTBFYV
FA8C1    WSTK---BPFS---WIKVDLLAPMIIHGIKTQGA--RQKFSSLYISQFII
A5C1     WT-PGBDT---VKBWIQVDLBNLRFVSGIGYQGAISKETKKKYFVKSYKV
A5C2     WALPPSNTHPYTKBWLQIDLABBKIVRGVIIQGG--KHKBNKVFMRKPKI
DDRC1    WC-PAGSVFPKBBBYLQVDLQRLHLVALVGTQGRHAGGLGKB-FSRSYRL
DISC     WC----SSIVDTNQYIVAGCBVPRTFMCVALQGR-GDADQW---VTSYKI
GP55
BAND16                     KMXVTXVVTQGA--SR
```

```
BA46C1   AYSLNGHBFD-PIHDVNKKHKBFVGNWNKNAVHVNLFBTPVBAQYVRLYP
MFGE8C1  AYSLDGRKFB-FIQDBSGGDKBPLGNLDNNSLKVNMPNPTLBABYIRLYP
BA46C2   AYSNDSANWTBYQDPRTGSSKIPPGNWDNHSHKKNLFBTPILARYVRILP
MFGE8C2  AHSDDGVQWTVYBBQ--GSSKVPQGNLDNNSHKKNIFBKPPMARKVRVLP
FA5C2    HYSBQGVBWKPYRLKSSMVDKIFBGNTNTKGHVKNPFNPPIISRFIRVIP
FA8C2    SSSQDGHQWTLPPQN--GKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHP
FA5C1    AYSSNQINWQIFKGNSTRNVMYPNGNSDASTIKBNQFDPPIVARYIRISP
FA8C1    MYSLDGKKWQTYRGNSTGTLMVPPGNVDSSGIKHNIFNPPIIARYIRLHP
A5C1     DISSNGBDWITLKGNKHL--VFTGNTDATDVVYRPFSKPVITRFVRLRP
A5C2     GYSNNGTBWBMIMDSSKNKPKTFBGNTNYDTPBLRTFAH-ITTGFIRIIP
DDRC1    RYSRDGRRWMGWKRWGQ--BVISGNBDPBGVVLKLGPPMVARLVRFYP
DISC     RYSLDNVSWPBYRNGAA-----VTGVTDRNTVVNHFFDTPIRARSIAIHP
GP55                               LNMFSAPLBVQYVR
BAND16                              INLFDTPLBTQYVR
```

**Table 6:**     Comparison of Deduced 46 Kdalton H M F G
Antigen Sequence to other Protein Sequences (Cont'd)

```
BA46C1    TS-CHTACTLRFELLGCE---LN           (SEQ. ID No:10)
MFGE8C1   VS-CHRGCTLRFELLGCE---LH           (SEQ. ID No:11)
BA46C2    VA-WHNRIALRLELLGC------           (SEQ. ID No:12)
MPGE8C2   VS-WHNRITLRLELLGC------           (SEQ. ID No:13)
FA5C2     KT-WNQSITLRLELFGC---DIY           (SEQ. ID No:14)
FA8C2     QS-WVHQIALRMEVLGCEAQDLY           (SEQ. ID No:15)
FA5C1     TR-AYNRPTLRLELQGCEVN---           (SEQ. ID No:16)
FA8C1     TH-YSIRSTLRMELMGCDLNS--           (SEQ. ID No:16)
A5C1      VT-WENGISLRFELYGCKI-TDY           (SEQ. ID No:17)
A5C2      ERASASGLALRLELLGCEVETPT           (SEQ. ID No:18)
DDRC1     RADRVMSVCLRVELYGC------           (SEQ. ID No:19)
DISC      LT-WNGHISLRCEFYTQ-                (SEQ. ID No:20)
GP55                     FELLGCE---LNGCLEPL  (SEQ. ID No:21)
BAND16    VELLGC                            (SEQ. ID No:22)
```

| | | | | | | |
|---|---|---|---|---|---|---|
| BA46C1: | Human 46 Kdalton | Milk Fat | Globule | Polypeptide | | (Clone 1) |
| BA46C2: | " | " | " | " | " | " | (Clone 2) |
| MFGE8C1: | Murine Milk Fat | Globule | Antigen | Polypeptide | | (Clone 1) |
| MFGE8C2: | " | " | " | " | " | " | (Clone 2) |
| FA5C1: | Coagulation | Factor | V | | | (Clone 1) |
| FA5C2: | " | " | V | | | (Clone 2) |
| FA8C1: | " | " | VIII | | | (Clone 1) |
| FA8C2: | " | " | VIII | | | (Clone 2) |
| A5C1: | A5 | Protein | | | | (Clone 1) |
| A5C2: | A5 | Protein | | | | (Clone 2) |
| DDRC1: | DDR | Protein | | | | (Clone 1) |
| DISC: | Discoidin | | Protein | | | |
| GP55: | | Protein | | | | |
| BAND16: | | | Protein | | | |

The difference between the human 46 Kdalton polypeptide (BA46) and MFG-E8 is that the former has a single EGF-like sequence and lacks the proline rich region that is present between the second EGF-like sequence and the C1C2-like sequence of coagulation factors V and VIII in MFG-E8 (See, Table 6 above). The cell adhesion

5     sequence RDG is also present in MFG-E8 but was not noted by the authors when published (Stubbs et al., supra), which is separated by the EGF-like sequence in both mouse and human proteins by 6 amino acids (See, Table 6 above). All cysteines in the human 46 Kdalton polypeptide are in identical positions compared to MFG-E8, two in the signal peptide, 3 preceding the RGD sequence, 3 in the EGF-like sequences, and

10    5 in the C/C2-like sequence. Both proteins have 4 N-linked glycosylation sites, but in the human 46 Kdalton polypeptide all are present in the C1C2-like sequence, while in MFG-E8, three are in the C1C2-like sequence and one after the first EGF-like sequence that is absent from the 46 Kdalton polypeptide. The second N-linked glycosylation site on the human 46 Kdalton polypeptide occurs is in the same position as in MFG-E8.

15       The cell adhesion sequence (RGD) was originally found in fibronectin and shown to be crucial for interaction with its cell surface receptor, such as the integrins (Cherny

et al, J. Biol. Chem. 268:9725 (1993)). Other proteins containing this cell adhesion sequence (RGD) are fibrinogen, vitronectin, Von Willebrand coagulation factor, entactin, some isoforms of tumor growth factor beta, and slime mold discoidin I (Poole et al., "Sequence and expression of the discoudin I gene family in dictyostelium", J. Mol. Biol.

5      153:273-289 (1981)).        The RGD sequence is also found on some collagens and on surface proteins of some animal viral proteins that serve the same cell adhesion purpose. Viruses whose surface proteins contain the RGD sequence include the coxsackie virus, the foot-and-mouth disease virus, the human immunodeficiency virus type 1 (HIV1), and certain flaviviruses such as Murray Valley encephalitis virus, the

10     Japanese encephalitis virus, the yellow fever virus, the West Nile virus Dengue type 4 virus, and the tick-borne encephalitis virus. The interaction of the cell adhesion sequence with its cell receptor is inhibited by synthetic peptides containing the RGD peptide.

        The function of the EGF-like sequences is not known. However, this sequence

15     is present on a number of growth factors, proteins associated with cell interaction and adhesion, and developmental proteins. The growth factors include TGF-alpha, amphiregulin, and growth factor-related proteins of the vaccinia, myxoma, and shope fibroma viruses. The EGF-like sequence is also present on coagulation associated proteins, complement components, fibronectin, selectins, and several Drosophila

20     developmental proteins such as the Notch-1, the neurogenic repetitive locus proteins, 95F and delta).

        The C1C2 domain of human coagulation factors V and VIII has been shown to be involved in phospholipid binding, that is an essential property for the involvement of these factors in coagulation. The binding appears to be to phosphatidyl serine (Ortel

25     et al., "Deletion analysis of recombinant human factor V. Evidence for a phosphotidyl serine binding site in the second C-type domain", J. Biol. Chem. 267:4189-4198 (1992)). The 46 Kdalton polypeptide appears to be a member of a family of proteins that contain these C-type domains (See, Table 6). These include the mouse milk fat globule protein MFG-E8 (Stubbs et al., supra), a putative neuronal cell adhesion

30     molecule (A5 antigen) of Xenopus larvis (Takagi et al., "The A5 Antigen, a candidate for the neuronal recognition molecule, has homologies to complement components and coagulation factors", Neuron 7: 295-307 (1991)), a receptor tyrosine kinase found in human breast carcinoma (DDR), and discoidin I, an endogenous lectin of slime mold Dictyostellum discoideum (Poole et al., supra). The coagulation factors, BA46, MFG-

35     E8, and the A5 antigen have two C-type domains that apparently resulted from an earlier tandem duplication. Both DDR and discoidin I have single C-type domains and

53

appear to be primitive members of the family.

Example 21:          Dendrogram of Protein Family

        A dendrogram of the alignment of the C-type domains shown in Table 6 above
was constructed and shows that this alignment likely occurred and split human protein
5    DDR and slime mold discoidin I from the other proteins with C and C2 domains. The
dendrogram is shown in Figure 2 accompanying this patent.

        A separation of the Xenopus A5 antigen from the other human coagulation
factors and the mouse and human milk fat globule proteins appears to have occurred
thereafter.  Finally, the coagulation factors were later separated from the milk fat
10   globule proteins.  The C1C2 domain appears to have evolved as a unit, since the C
regions of BA46 and MFG-E8 have more homology than with their own C2 domains.
This is also the case for the C and C2 domains of factors V and VIII (See, Table 6
above).  In fact, the C2 domains of the coagulation factors are more homologous to
the BA46 and MFG-E8 C2 domains than they are to the their own C domains.  The
15   similarity also extends to the sequences of the peptides from the milk fat globule
components of bovine (component 16) and guinea-pig (GP-55) (Mather et al., "The
major fat-globule membrane proteins, bovine components 15/16 and guinea-pig GP 55,
are homologous to MGF-E8, a murine glycoprotein containing epidermal growth factor-
like and factor V/VIII-like sequences", Biochem. Mol. Biol. Int. 29: 545-554, 1993)).
20   (See, Table 6 above).

        The C2 domain of the coagulation factors is critical for phospholipid binding.
This is also most likely the case for the C2-like domains of the milk fat globule factors,
including the 46 Kdalton  HMFG polypeptide of the invention.  The current evidence
suggests that the MFG-E8 antigen binds to phospholipids, and that this binding is $Ca^{++}$
25   dependent (Buse et al, J. Cell Biol.  115:1969 (1991)).  In contrast, the replacement
of the C2 domain of factor V with the C2-like domain of the 46 Kdalton  HMFG
polypeptide was shown to abolish the phospholipid binding properties in the chimeric
protein, while its replacement with the C2 domain of factor VIII did not (Ortel et al.,
"Epitope mapping of the C2 domain of coagulation factor V using antibodies and
30   chimeras with heterologous C-type domains" (1993)).  In contrast to the phospholipid
binding reported by Parry et al, supra, this binding is not $Ca^{++}$ dependent.

        These results permit the grouping of the 46 Kdalton  HMFG polypeptide with
growth factors and other molecules associated with cell adhesion\interactions, e.g.
associated with breast epithelial cells, that provide a possible autocrine/paracrine
35   function.  The 46 Kdalton  HMFG antigen is thus  related to selectin-like molecules (

Larigan, J.D., Tsang, T.C., Rumberger, J.M., and Burns, D.K., "Characterization of cDNA and genomic sequences encoding rabbit ELAM-1: Conservation of structure and functional interactions with leukocytes", DNA Cell Biol. 11:149-162 (1992)), which have the general structure of an N-terminal adhesion domain (lectin domain) followed

5   by an EGF-like domain, a variable number of complement regulatory elements, a membrane attachment domain (a single transmembrane sequence) and a short cytoplasmic tail ( Larigan, J.D., Tsang, T.C., Rumberger, J.M., and Burns, D.K., "Characterization of cDNA and genomic sequences encoding rabbit ELAM-1: conservation of structure and functional interactions with leukocytes", DNA Cell Biol.

10   11:149-162 (1992)). The C-type domain is very likely the means by which the 46 Kdalton HMFG polypeptide associates with the cell membrane by interaction with phospholipids. The possible cell adhesion properties may be mediated via the cell adhesion sequence RGD since breast cells are known to possess integrins that have receptors for this sequence. The autocrine may be mediated by the EGF-like sequence.

15   The 46 Kdalton HMFG antigen is abundantly present in the HMFG and the expression of its mouse homologue is increased during lactation (Stubbs et al., supra). Thus, the expression of the human 46 Kdalton HMFG antigen and its mouse homologue appear to be associated with differentiation in the breast.

The overexpression of the 46 Kdalton antigen mRNA in some breast, lung and

20   ovarian cancers such as carcinomas shows that the 46 Kdalton antigen is expressed with other epithelial tissues and may be deregulated in malignancy. Its expression in breast cancers such as carcinomas makes it a good target for monoclonal antibody therapy. Of the known monoclonal antibodies raised against the 46 Kdalton HMFG antigen, Mc3, which recognizes an epitope in the N-terminal region of the polypeptide,

25   is more effective in radioimmunotherapy than Mc8, which recognizes an epitope in the C2-like domain of the 46 Kdalton polypeptide (unpublished results). This effectiveness of Mc3 in radioimmunotherapy is in all likelihood the result of an internalization of the antigen-antibody complex formed, which increases the residence time of the radiolabel in the tumor. In addition, the antibody is likely involved in modulating cell growth by

30   interfering with the effect of the target antigen on growth regulation and cell association.

The anti-viral activity of the 46 Kdalton HMFG polypeptide appears to be mediated via binding of the antigen to the virus (Yolken et al., "Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis", J. Clin. Invest.

35   90: 1984-1991 (1992)). The desialylation of the 46 Kdalton HMFG polypeptide was shown to abolish its anti-viral activity (Yolken et al., supra.)

55

**Example 22:**          **Bacterial Expression of Complete**
                         **46   Kdalton   H M F G   Antigen**

A recombinant polynucleotide and its polypeptide product encompassing the entire 46 Kdalton HMFG antigen were produced by cloning the BA46 cDNA segment

5   that codes for entire ORF except for the signal peptide obtained above.   Poly A containing mRNA was isolated from a breast cell line (ELL-G) using the FastTrack mRNA isolation kit (Invitrogen, San Diego) according to the manufacturer's instructions. The mRNA was reversed transcribed and amplified using an upstream primer at the 3' end of the signal peptide encoding sequence and a downstream primer

10  at the first stop codon.   Each primer was constructed to have a Hind III restriction enzyme site. The amplified sequence was then cut with the restriction enzymes Stu I and Apa I, which cut it into three fragments. These fragments were cloned into a pBS vector and sequenced to verified that no mutations were introduced by the PCR reactions. Once the identity of the sequence fragments was verified, the fragments

15  were ligated together and cloned into the pBR322/lacP/OmpA and pBS//OmpA expression vectors. The expression of the product in the vector was thus driven by the LacP promoter and and a bacterial signal peptide substituted for the BA46 signal peptide. Both vectors, when transfected into E. coli, expressed the 46 Kdalton HMFG antigen. The presence of the product was observed in the medium, periplasm, and

20  protoplast of the transfected E. coli. The authenticity and identity of the recombinant peptide produced was demonstrated by binding of all available anti-46 Kdalton HMFG antigen monoclonal antibodies. Mc3, Mc8, Mc15, Mc16, to the transfected E. coli extracts in a solid phase radioimmunobinding assay.   All the monoclonal antibodies recognized epitopes on the peptide core of the 46 Kdalton  HMFG antigen.   No

25  monoclonal antibody bound to control bacteria extracts transfected with the same vectors but without the insert.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

## CLAIMS

1.	A purified, isolated polypeptide having the antibody binding specificity of the 46 Kdalton apparent molecular weight (app. MW) human milk fat globule (HMFG) antigen and/or homology to at least a portion of one of the light chains of clotting factors V and VIII and/or comprising an RGD and/or EGF-like segment.

2.	The polypeptide of claim 1, being the 46 Kdalton HMFG app. MW antigen or a binding fragment thereof.

3.	The polypeptide of claim 1, having the biological activity of the 46 Kdalton HMFG antigen.

4.	The polypeptide of claim 1, having an estimated MW of about 43,123 or about 387 amino acids.

5.	The polypeptide of claim 1, having an estimated MW of about 40,862 or about 365 amino acids.

6.	The polypeptide of claim 1, having the amino acid sequence shown in Table 4 (SEQ. ID No: 6) or a binding fragment thereof of about 5 to 100 amino acids.

7.	The polypeptide of claim 6, having the amino acid sequence shown in Table 2 (SEQ. ID NO: 3) or a binding fragment thereof of about 5 to 100 amino acids.

8.	The polypeptide of claim 1, in glycosylated form.

9.	A composition comprising an antibody binding effective amount of the polypeptide of claim 1, and a biologically acceptable carrier.

10.	A pharmaceutically composition comprising the composition of claim 9, wherein the carrier comprises a pharmaceutically-acceptable carrier.

11.	A fusion protein, comprising the polypeptide of claim 1, and a second polypeptide or a binding fragment thereof bound thereto.

12.	A composition comprising the fusion protein of claim 11, and a diluent or biologically acceptable carrier.

13.	The pharmaceutical composition of claim 12, wherein the carrier comprises a pharmaceutically-acceptable carrier.

14.	An in vitro assay for detecting the presence in a biological sample of the 46 Kdalton HMFG antigen or fragments thereof, comprising obtaining a biological sample suspected of comprising the antigen or a fragment thereof; adding thereto an antibody selectively binding the antigen and a known labeled amount of the polypeptide of claim 1 under conditions effective to form antibody-polypeptide and antibody-antigen complexes; determining the amount of labeled antibody-polypeptide complex formed;

and comparing the result with a control conducted without the sample.

15. An in vitro assay for determining the presence of a cancerous tumor of epithelial origin, comprising obtaining a biological sample from a subject suspected of being afflicted with a cancer of epithelial origin; adding thereto an antibody selectively binding the 46 Kdalton app. MW HMFG antigen and a known labeled amount of the polypeptide of claim 1 under conditions effective to form labeled antibody-polypeptide and antibody-cell antigen complexes; determining the amount of labeled complex formed; and comparing the result to a control without the sample.

16. The composition of claim 42 in a form suitable for imaging neoplastic tumors of epithelial origin, comprising the anti-46 Kdalton HMFG antigen antibodythe antobody in an amount effective to deliver it to the area of a subject's body containing malignant tumor cells of epithelial origin to form an antibody-cell polypeptide complex, which can be detected by addition of a label capable of binding to the antibody at a site other than the polypeptide.

17. The composition of claim 10, comprising an amount of the polypoeptide effective to elicit an immunologic response suitable for vaccination against neoplastic tumors of epithelial origin.

18. An in vitro assay for diagnosing the presence of a neoplastic tumor of epithelial origin, comprising obtaining a sample from a subject suspected of being afflicted with a neoplastic tumor of epithelial origin; adding thereto the polypeptide of claim 1 under conditions effective to form antibody-46 Kd HMFG antigen and antibody-antigen fragment complexes; and determining the presence of any complexes formed.

19. An in vitro assay for diagnosing a neoplastic tumor of epithelial origin, comprising obtaining a sample from a subject suspected of being afflicted with a neoplastic tumor; adding thereto an the fusion protein of claim 10 under conditions effective to form an antibody-fusion protein complex; adding thereto the anti-second polypeptide antibody under conditions effective to form a double antibody-fusion protein complex; and determining the presence of any double antibody-fusion protein complex formed.

20. A composition suitable for administration to a subject suspected of carrying a neoplastic tumor of epithelial origin to deliver a therapeutic agent to target tumor cells of epithelial origin, comprising a therapeutically effective amount of a therapeutic agent bound to the antibody of claim 41 at a site other than the antigen binding site under conditions effective for the antibody to bind to the tumor cells' polypeptide and allow the therapeutic agent to exert its effect on the cells.

21.    An ex vivo method of delivering a therapeutic agent to target neoplastic tumor cells,  comprising obtaining a biological sample from a subject suspected of being afflicted with a neoplastic tumor of epithelial origin; binding a therapeutic agent to the antibody of claim 41 at a site other than the antigen binding site; adding the antibody-bound therapeutic agent to the sample under conditions effective to promote the formation of an antibody-cell polypeptide complex; allowing the agent to exert its effect on the cells; and returning the sample to the subject.
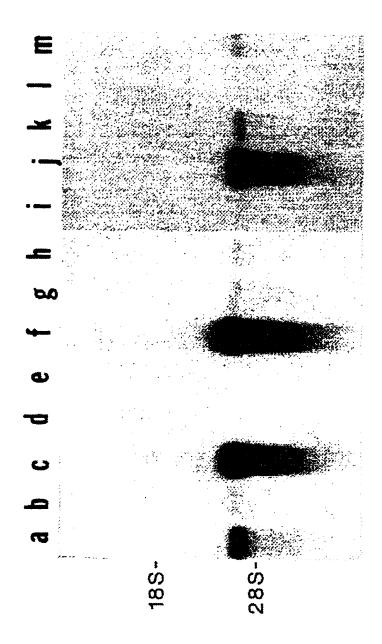
22.    The composition of claim 10 in a form suitable for vaccination of mammals against neoplastic tumors, comprising an antigenically effective amount of the polypeptide.

23.    A polyribonucleotide comprising an oligoribonucleotide encoding the polypeptide of claim 1 or 5 to 100 amino acid fragments thereof.

24.    A polydeoxynucleotide comprising a DNA segment having a nucleotide sequence complementary to that of the polyribonucleotide of claim 23.

25.    The polyribonucleotide of claim 23, being a polydeoxyribonucleotide comprising the DNA sequence shown in Table 4 (SEQ. ID No: 7) or fragments thereof encoding 5 to 100 amino acids thereof.

26.    The polyribonucleotide of claim 25, having the DNA sequence shown in Table 1 (SEQ. No. 1) or fragments thereof encoding fragments 5 to 100 amino acids thereof.

27.    The polyribonucleotide of claim 23, in labeled form.

28.    The polyribonucleotide of claim 25, being an RNA.

29.    The polydeoxyribonucleotide of claim 28 in labeled form.

30.    A polyribonucleotide comprising an oligoribonucleotide encoding the fusion protein of claim 11.

31.    An in vitro assay for diagnosing a neoplastic tumor of epithelial origin, comprising obtaining a biological sample from a subject suspected of being afflicted with a neoplastic tumor of epithelial origin; lysing any cells comprised in the sample to expose polynucleotides contained therein; adding thereto the polynucleotide of claim 24 in single stranded form under stringent conditions to hybridize any polynucleotides having a complementary sequence thereto of about at least 15 bases; and detecting the presence of any polynucleotide-DNA hybrid.

32.    An in vitro assay for diagnosing a neoplastic tumor of epithelial origin, comprising obtaining a sample from a subject suspected of being afflicted with a neoplastic tumor of epithelial origin; lysing any cells comprised in the sample to expose polynucleotides contained therein; adding thereto the polynucleotide of claim
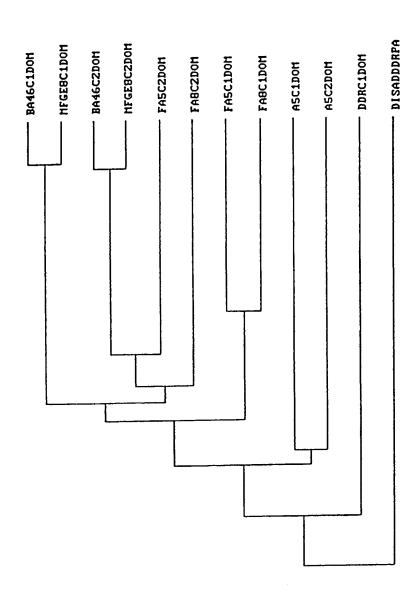
30, in DNA labeled form, under stringent conditions to hybridize any polynucleotides having a complementary sequence thereto of at least about 15 bases; and detecting the presence of the polynucleotide-DNA hybrid.

33.     A DNA segment comprising an anti-sense sequence to the coding strand of the polyribonucleotide of claim 23 of about 15 to 3000 nucleotides.

34.     A pharmaceutical composition, comprising the anti-sense DNA sequence of claim 33, and a pharmaceutically-acceptable carrier.

35.     The composition of claim 34 comprising a therapeutically effective amount of the anti-sense DNA segment, for treating a neoplastic tumor of epithelial origin.

36.     The composition of claim 35, for administration by a parenteral, intravenous, or intracavitary or other localized route.

37.     An antibody detecting kit comprising, in separate containers, the polypeptide of claim 1; anti-constant region immunoglobulin, protein G or A or binding fragments thereof; and instructions for its use.

38.     The kit of claim 37, further comprising anti-46 Kdalton HMFG antigen antibody or fragments thereof.

39.     A fusion protein kit comprising, in separate containers, the fusion protein of claim 11; anti-46 Kdalton HMFG antigen monoclonal antibody; anti-second polypeptide monoclonal antibody; anti-constant region immunoglobulin, protein G or A or binding fragments thereof; and instructions for its use.

40.     Antibody raised against, and selectively binding, the 46 Kdalton app. MW HMFG antigen, the antibody having an affinity constant for the antigen of about $10^{10}$ to $10^5$ $M^{-1}$.

41.     The antibody of claim 40, being a monoclonal antibody.

42.     A composition, comprising the antibody of claim 40, and a non-proteolytic carrier.

43.     An anti-cancer therapeutic kit, comprising, in separate containers, the monoclonal antibody of claim 40; an anti-cancer therapeutic agent selected from the group consisting of immunotoxins and radionucleides, which agent is bound to the antibody; and instructions for use of the kit.

44.     A specifically targeted anti-cancer agent, comprising the antibody of claim 40, and an anti-cancer agent operatively linked thereto.

45.     An anti-cancer kit, comprising the specifically targeted anti-cancer agent of claim 44; and instructions for its use.

FIGURE 1

1 / 2

FIGURE 2

2 / 2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13967

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/277.1; 435/7.1, 69.3; 530/350, 387.7, 388.15, 828; 536/23.4, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, EMBASE, DERWENT
search terms: HMFG, antigen, 46 Kd

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X -- Y | Proceedings of the National Academy of Sciences USA, Volume 79, issued September 1982, R.L. Ceriani et al., "Circulating Human Mammary Epithelial Antigens in Breast Cancer", pages 5420-5424, especially page 5420. | 16, 20-21, 40-42 ---------------- 43-45 |
| X -- Y | Cancer Research, Volume 51, issued 15 September 1991, D. Larocca et al., "A $M_r$ 46,000 Human Milk Fat Globule Protein that is Highly Expressed in Human Breast Tumors Contains Factor VIII-Like Domains", pages 4994-4998, see entire document. | 23, 24, 27 ---------------- 1-3, 8-22, 30-45 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| * | Special categories of cited documents: |
|---|---|
| "A" | document defining the general state of the art which is not considered to be of particular relevance |
| "E" | earlier document published on or after the international filing date |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) |
| "O" | document referring to an oral disclosure, use, exhibition or other means |
| "P" | document published prior to the international filing date but later than the priority date claimed |

| | |
|---|---|
| "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "&" | document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 08 MARCH 1995 | **16 MAR 1995** |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | MICHAEL S. TUSCAN |
| Facsimile No. (703) 305-3230 | Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)★

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| Y | Analytical Biochemistry, Volume 201, Number 1, issued 15 January 1992, R.L. Ceriani et al., "A Novel Serum Assay for Breast Epithelial Antigen Using a Fusion Protein", pages 178-184, see entire document. | 13-16, 30, 32, 39 |
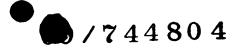
# INTERNATIONAL SEARCH REPORT

**Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:  4-7, 25, 26, 28 and 29
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

   no computer readable form of the disclosed sequences was provided.  Without a computer readable sequence listing
   for the  claimed DNA, no electronic search of the available sequence data bases could be performed.

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
   claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers
   only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant.  Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)★

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/20, 35/55, 39/00, 39/395; G01N 33/53; C07K 14/435, 16/18, 16/30; C12N 15/12, 15/62

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

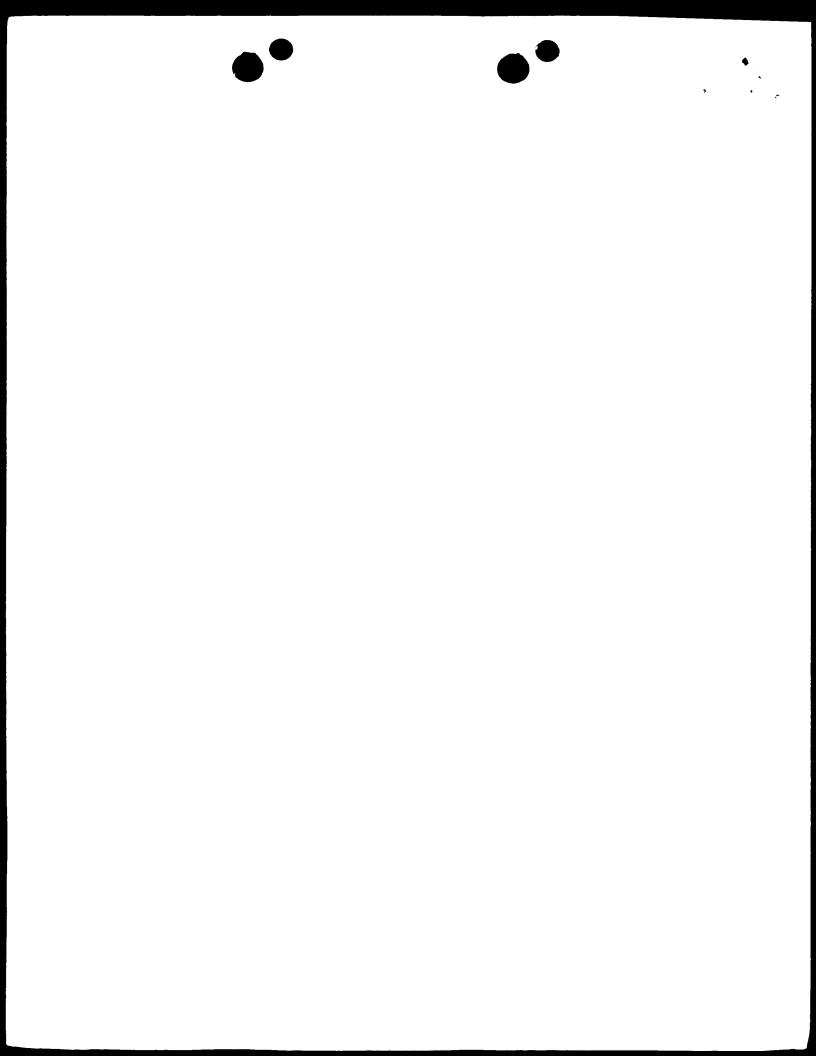424/277.1; 435/7.1, 69.3; 530/350, 387.7, 388.15, 828; 536/23.4, 23.5

/744804

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

## (PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br><br>453-A-PCT | **FOR FURTHER ACTION** | See Notification of Transmittal of International<br>Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/IL99/00417 | International filing date *(day/month/year)*<br>29/07/1999 | Priority date *(day/month/year)*<br>30/07/1998 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/12 | | |
| Applicant<br><br>YEDA RESEARCH AND DEVELOPMENT COMPANY ... et al. | | |

1.  This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2.  This REPORT consists of a total of 12 sheets, including this cover sheet.

    ☒  This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

    These annexes consist of a total of 6 sheets.

3.  This report contains indications relating to the following items:

    I     ☒   Basis of the report
    II    ☐   Priority
    III   ☒   Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
    IV   ☒   Lack of unity of invention
    V    ☒   Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
    VI   ☒   Certain documents cited
    VII   ☐   Certain defects in the international application
    VIII   ☒   Certain observations on the international application

| Date of submission of the demand<br><br>17/01/2000 | Date of completion of this report<br><br>10.11.2000 |
|---|---|
| Name and mailing address of the international<br>preliminary examining authority:<br><br>    European Patent Office<br>    D-80298 Munich<br>    Tel. +49 89 2399 - 0  Tx: 523656 epmu d<br>    Fax: +49 89 2399 - 4465 | Authorized officer<br><br>Armandola, E<br><br>Telephone No. +49 89 2399 7493 |
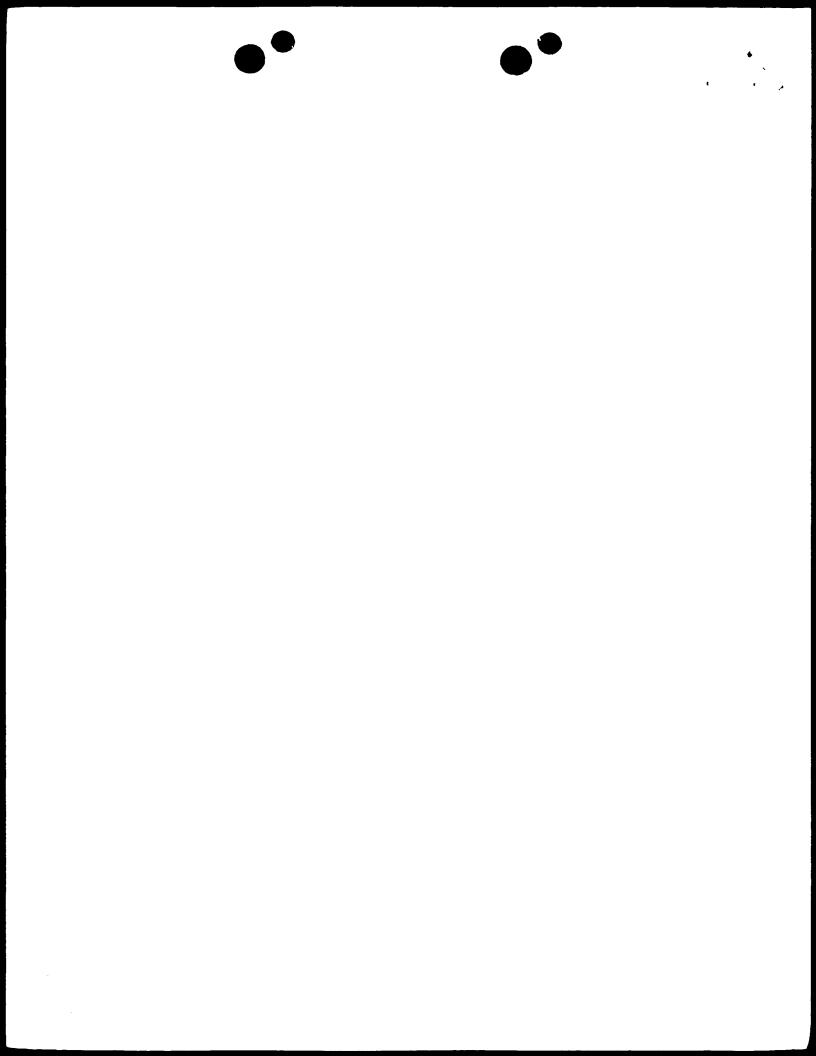
Form PCT/IPEA/409 (cover sheet) (January 1994)

## INTERNATIONAL PRELIMINARY
## EXAMINATION REPORT

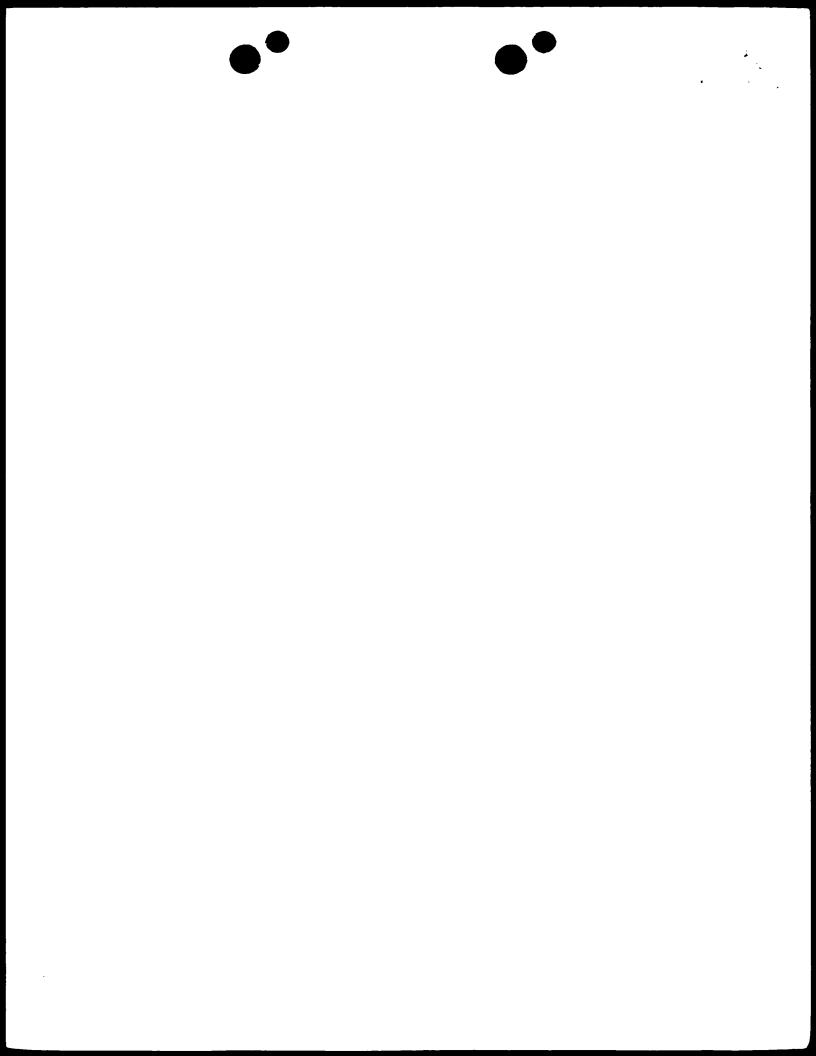International application No.  PCT/IL99/00417

### I.  Basis of the report

1.  This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
**Description, pages:**

| | |
|---|---|
| 1-53 | as originally filed |

**Claims, No.:**

| | | |
|---|---|---|
| 1-52 | with telefax of | 05/10/2000 |

**Drawings, sheets:**

| | |
|---|---|
| 1/29-29/29 | as originally filed |

2.  With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language:    , which is:

☐  the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐  the language of publication of the international application (under Rule 48.3(b)).

☐  the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3.  With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐  contained in the international application in written form.

☐  filed together with the international application in computer readable form.

☐  furnished subsequently to this Authority in written form.

☐  furnished subsequently to this Authority in computer readable form.

☐  The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐  The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4.  The amendments have resulted in the cancellation of:

☐  the description,      pages:

☐  the claims,      Nos.:

☐   the drawings,         sheets:

5. ☐   This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

## III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐   the entire international application.

   ☒   claims Nos. 21-23 (partially) and 41-43 (complete) (N, IS, IA), 35, 36, 49-51 (IA).

because:

   ☒   the said international application, or the said claims Nos. 35, 36, 49-51 relate to the following subject matter which does not require an international preliminary examination *(specify)*:
   **see separate sheet**

   ☐   the description, claims or drawings *(indicate particular elements below)* or said claims Nos.  are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐   the claims, or said claims Nos.  are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒   no international search report has been established for the said claims Nos. 21-23 (partially) and 41-43 (complete).

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐   the written form has not been furnished or does not comply with the standard.
   ☐   the computer readable form has not been furnished or does not comply with the standard.

## IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐   restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
**see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 2-4, 6, 8, 10-18, 38-40 |
| | No: | Claims | 1, 5, 7, 9, 19-37, 44-52 |
| Inventive step (IS) | Yes: | Claims | 2-4, 10-18 |
| | No: | Claims | 1, 5- 9, 19-40, 44-52 |
| Industrial applicability (IA) | Yes: | Claims | 1-34, 37-48, 52 |
| | No: | Claims | |

2. Citations and explanations
**see separate sheet**
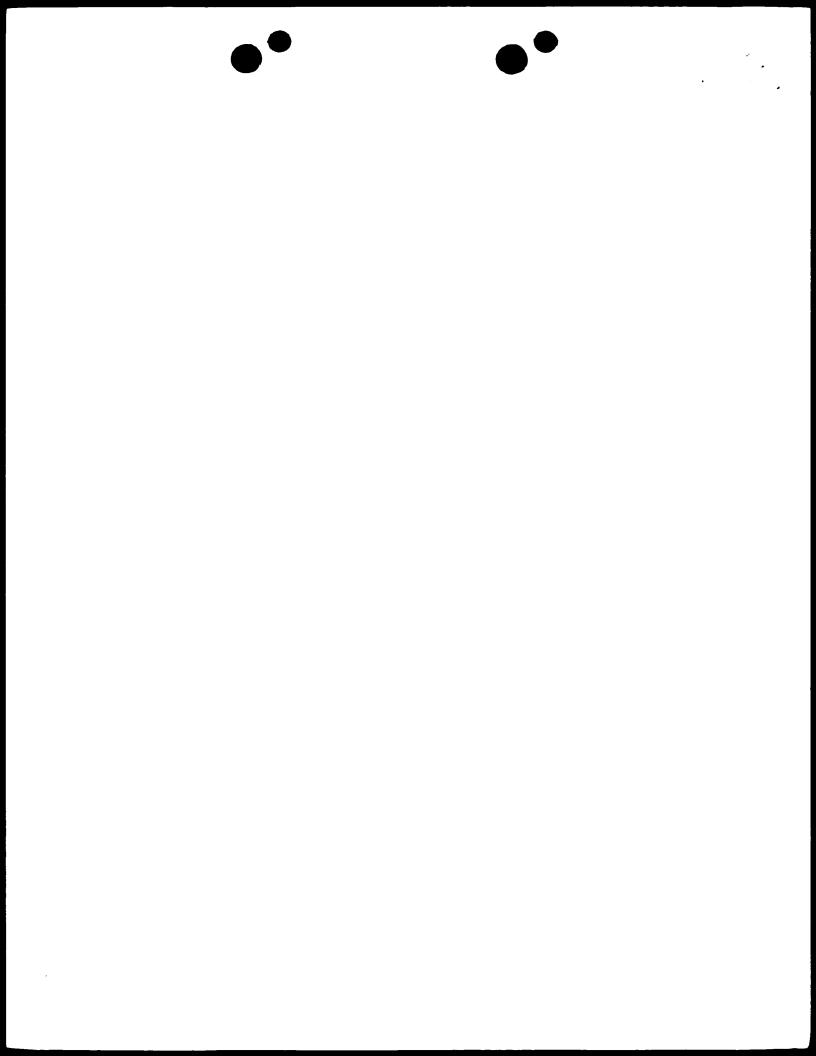
## VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

**see separate sheet**
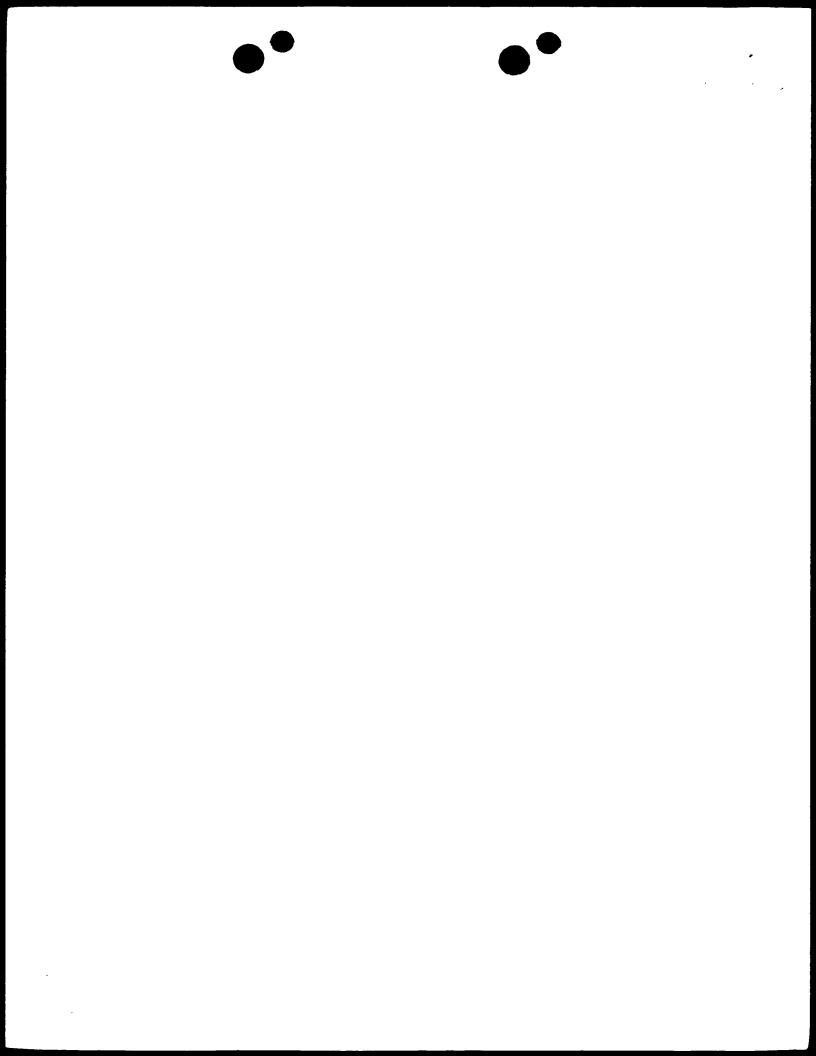
**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

## Re Item III
## Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Novelty, Inventive step and Industrial applicability (Art. 33(2)(3)(4) PCT)

i) Claims 21-23 were examined for novelty, inventive step and industrial applicability insofar as a search report has been established (see comments on ISR). As noted by the ISA, the claims relate to an extremely high number of possible compounds/products of which only the ones disclosed and supported by the description have been searched.

ii) Claims 41-43 were not examined with regard to novelty, inventive step and industrial applicability because no search report has been established for these claims due to insufficient characterization of their subject-matter (see ISR).

Industrial applicability (Art. 33(4) PCT)

Claims 35, 36 and 49-51 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(i) PCT).

For the assessment of the present Claims 35 and 36, with regard to methods of treatment of the human body, and 49-51 with regard to the medical use of a compound, on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claim. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

## Re Item IV
## Lack of unity of invention

The IPEA is of the opinion that the present application concerns more than one invention

and, therefore, lacks unity in view of Rule 13 PCT.

Seven separate groups of inventions have been identified:

1.    Claims 1 (partially), 2-4, 19-52 (partially).

A peptide derived from Uroplakin, where Uroplakin (UP) is selected from the group of Uroplakin II, Uroplakin Ia, Uroplakin III and Uroplakin Ib; the peptide having a sequence selected from SEQ. ID. NO: 1-19 and 50-64; the peptide with modifications; a pharmaceutical composition containing the peptide as active ingredient; the composition being effective in the prevention or cure of cancer; the composition being a vaccine; the composition additionally comprising a helper peptide; a method for the prevention or cure of cancer by administration of the composition to a patient; the use of the peptide as a medicament; a polynucleotide encoding the peptide;   a pharmaceutical composition comprising such polynucleotide.

2.    Claims 1 (partially), 5, 6, 19-52 (partially).

As 1., wherein the peptide is derived from Prostate specific antigen (PSA), the peptide having a sequence derived from SEQ. ID. NO: 21-23.

3.    Claims 1 (partially), 7, 8,19-52 (partially).
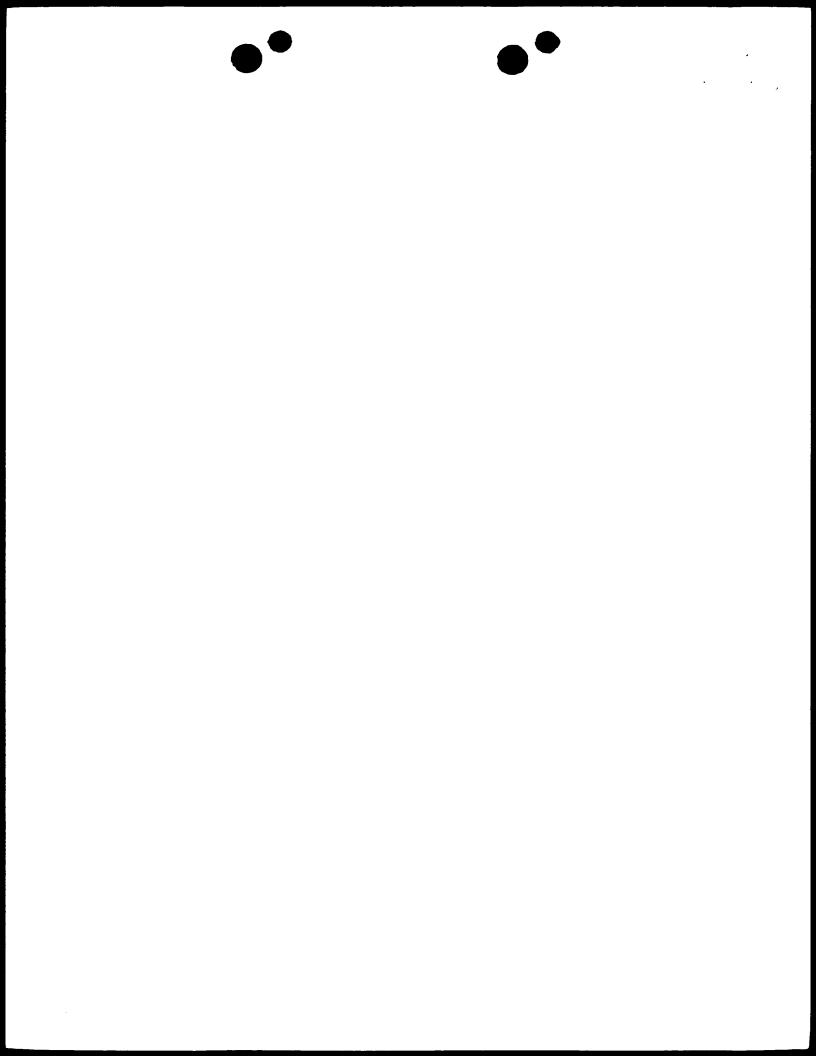
As 1., wherein the peptide is derived from Prostate specific membrane antigen (PSMA), the peptide having a sequence derived from SEQ. ID. NO: 28.

4.    Claims 1 (partially), 9,19-52 (partially).

As 1., wherein the peptide is derived from Prostate acid phosphatase (PAP).

5.    Claims 1 (partially), 10-14, 19-52 (partially).

As 1., wherein the peptide is derived from Mucin (MUC1), the peptide having a sequence derived from SEQ. ID. NO: 42-49.

6.    Claims 1 (partially), 15, 16,19-52 (partially).

As 1., wherein the peptide is derived from Lactadherin (BA-46), the peptide having a sequence derived from SEQ. ID. NO: 35-41.

7.    Claims 1 (partially), 17, 18,19-52 (partially).

As 1., wherein the peptide is derived from Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide having a sequence derived from SEQ. ID. NO: 66-77.

The seven groups of inventions are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

the proteins from which the peptides are derived as well as isolated peptides from these proteins were known. The only common feature between the various groups of inventions is that the claimed peptides are predicted to bind HLA-A2. Peptides binding to HLA-A2 were, however, known (see e.g. D1). No further technical feature can be identified which, in the light of the prior art, is common to the above listed group of inventions and constitutes a novel and inventive concept.

However, as the ISA has provided a search report for all the groups of inventions, this authority has decided not to invite the applicant to pay additional fees and to carry out the complete examination.
The objection of lack of unity will, however, be prosecuted during regional phase examination at the EPO.
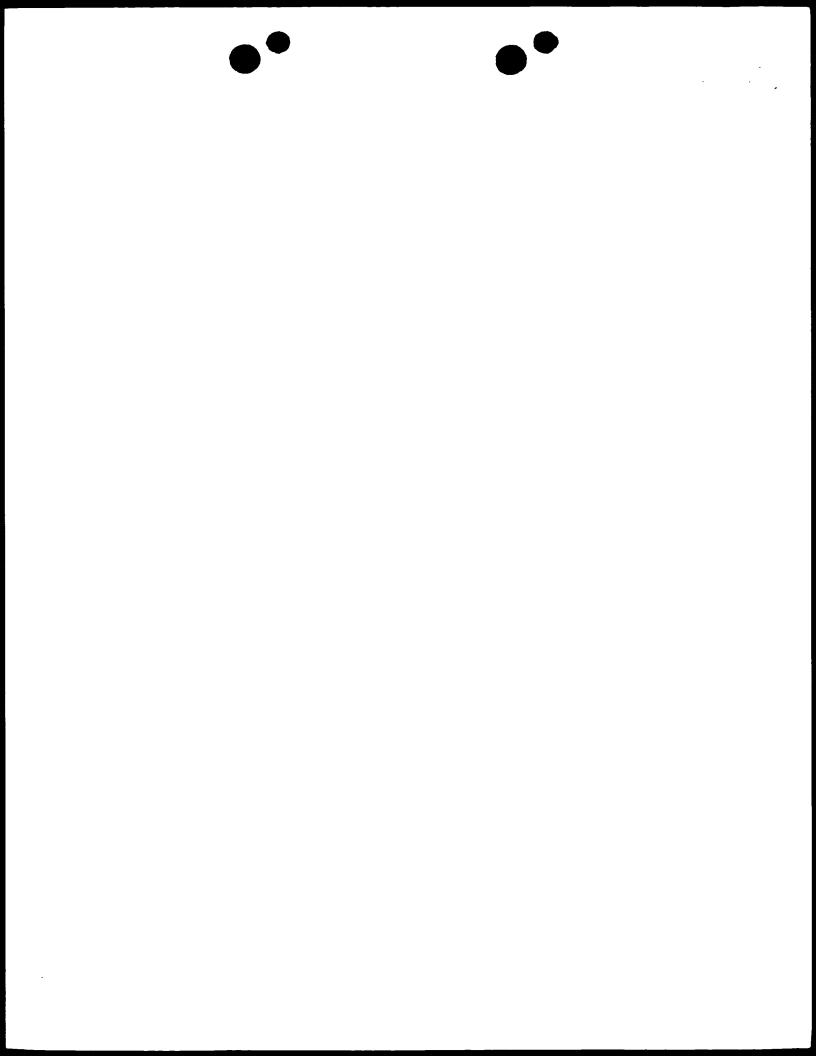
## Re Item V
**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Novelty and Inventive step (Art. 33(2)(3) PCT)

Reference is made to the following documents:

D1:   WO 94 20127 A (CYTEL CORP) 15 September 1994 (1994-09-15)

D2:   SALGALLER ML ET AL.: 'Report of Immune Monitoring of Prostate Cancer Patients Undergoing T-Cell Therapy Using Dendritic Cells Pulsed With HLA-A2-Specific Peptides From Prostate-Specific Membrane Antigen (PSMA)', THE PROSTATE, 01 May 1998, vol. 35, no. 2, pages 144-151

D3:   XUE B. ET AL.: 'Induction of human cytotoxic T lymphocytes specific for prostate-specific antigen.', THE PROSTATE, 1997, vol. 30, pages 73-78

D4:   PESHWA MV ET AL.: 'Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide.', THE PROSTATE, 01 July 1998, vol. 36, no. 2, pages 129-138

D2-D4 were not cited in the ISR.

D1 discloses peptides that bind to HLA-A2 and can induce T cell activation, eliciting an immune response to the antigen from which they are derived. Peptides derived from PSA and PAP are disclosed which are identical or overlap with peptides of the present application. D1 also describes compositions and methods for treating or diagnosing cancer comprising the above mentioned peptides or modified forms thereof linked or not to a carrier, vaccines and vaccination methods employing the peptides.
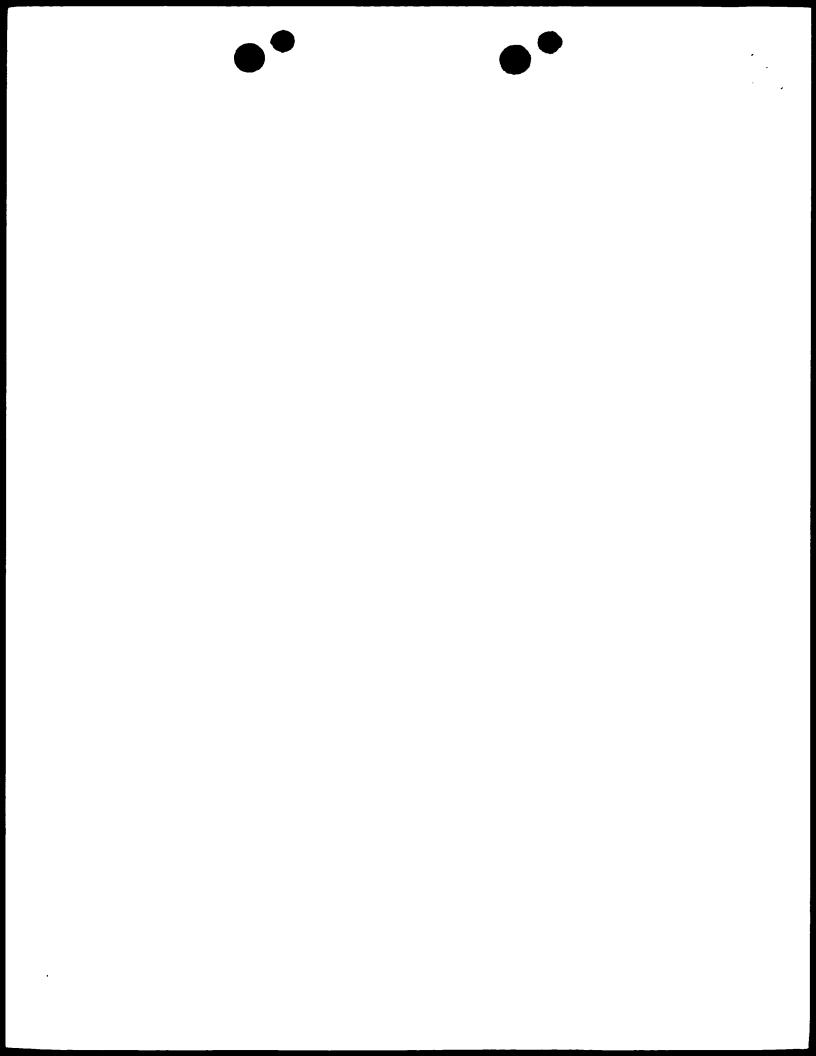
D2 reports the immune monitoring of prostate cancer patients undergoing a Phase II clinical trial in which they received autologous dendritic cells pulsed with PSMA peptides which binds to HLA-A2. Peptides identical to SEQ. ID. NO: 25-27, 29 and 30 of the application are disclosed.

D3 discloses the identification of two peptides derived from PSA which bind HLA-A2 and can stimulate specific CD8+ CTLs recognizing peptide loaded targets.

D4 reports the induction of prostate tumor-specific CTLs by dendritic cells coated with PAP peptides binding to HLA-A2. Among the peptides disclosed are peptides identical to SEQ. ID. NO: 31, 33 and 34 of the application.

Novelty and Inventive step (Art. 33(2)(3) PCT)

i) Claims 1, 5, 7, 9, 19-37, 44-52 cannot be considered novel in view of document D1, D2

and D4 which disclose peptides of PSA, PSMA and PAP (D1, p.87, Table 25; D2, p.147, Table I; D4, p.133, Table I) which fall under the scope of Claim 1 (8-10 amino acids, of which a second residue from the $NH_2$ terminus and the end residue at the COOH terminus are hydrophobic or hydrophylic). The peptides described in D1 are of human origin. Modifications of the peptides with non natural amino acids are also foreseen in D1 (see e.g. D1, p. 14, from line 25, p. 17, line 34).
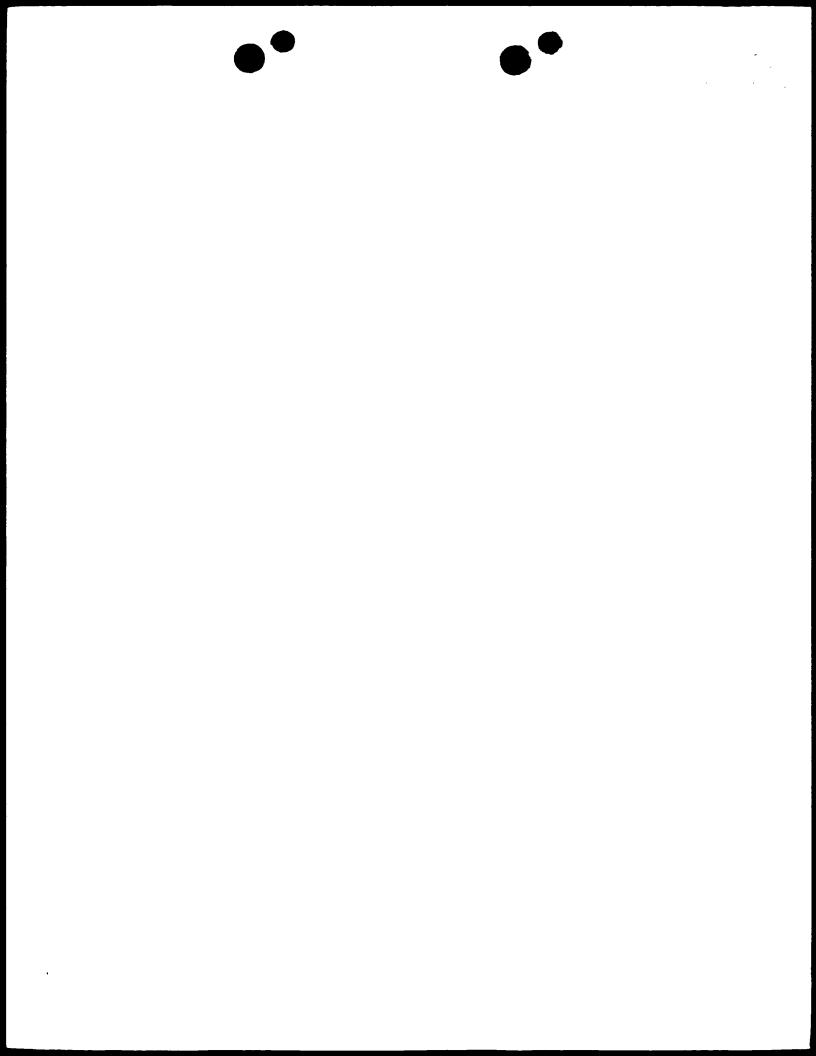
Pharmaceutical compositions and vaccines containing the peptides (without or with helper peptides) for prevention or cure of cancer are also disclosed (D1, Claims 1, 11, 19, 22; p. 26, lines 8-13). Methods of treatment employing the compositions and polynucleotides encoding the peptides are also described (D1, p. 21, last paragraph and top, respectively).

ii) Claims 6, 8, 38-40 can be considered novel because polynucleotides encoding the specific peptides disclosed in the claims, fusion proteins and pharmaceutical compositions containing them have not been disclosed. However, the claims cannot be considered to entail an inventive step for the following reasons:

Claims 6 and 8 refer to PSA and PSMA peptides which differ from the ones disclosed in D1 and D2. The problem to be solved can therefore be seen as the provision of additional peptides which present the structural features described in Claim 1. The skilled person would not need to exercise inventive activity to arrive at the peptide described in the claims by scanning of the protein for sequences fulfilling the simple requirements set in Claim 1, It should be noted that SEQ. ID. NO: 21 and 23 are identical to peptides described in D1 except for the addition or truncation of one amino acid.

D1 discloses several peptides from PSA and fusion proteins comprising one or more peptides, which are able to bind to HLA-A2 and to stimulate T-cells (D1, p.87, Table 25 and p. 21, lines 9-12). The subject-matter of Claim 38 includes peptides derived from PSA; the difference between the PSA peptides included in claim 38 and the disclosure of D1 is the slight length difference for the peptides with SEQ. ID. NO: 21 and 23. The skilled person is well aware of the possibility to vary peptide length of 1 or 2 amino acids and still maintain HLA binding and immunogenicity. Claim 39 refers to a fusion protein for which the only diifference with the disclosure of D1, with reference to PSA peptides, is the possibility to cleave the peptides from the fusion protein through a protease; the practice of adding a protease cleavage site to a fusion protein is also well known and frequently used by the skilled person when a (poly)peptide initially produced as a fusion product needs to be obtained in a pure form.

The provision of a composition containing a polynucleotide encoding not novel (Claim 37)

or not inventive (Claims 38 and 39) polypeptides cannot be considered to entail an inventive step as DNA vaccination with polynucleotides encoding immunogenic peptides is well known and widely used.

iii) Claims 2-4 and 10-18 can be considered novel and inventive. Peptides derived from Uroplakin, Lactadherin, Mucin and CRIPTO-1, binding to HLA-A2 , having the sequences represented by SEQ. ID. NO: 1-19, 35-41, 42-49, 50-64 and 66-77 have not been described in the available prior art.

Although the idea of analysing a protein which is expressed by a tumor for HLA-binding motifs in order to identify potentially immunogenic peptides for therapy is not new, no hints can be found in the prior art that would lead the skilled person to selecting the above mentioned proteins for the screening of potential HLA-A2 restricted epitopes to be used in tumor therapy. In addition a certain degree of uncertainty is still linked to the prediction of T-cell epitopes, which renders the selection of candidate peptides not trivial.

## Re Item VI
## Certain documents cited

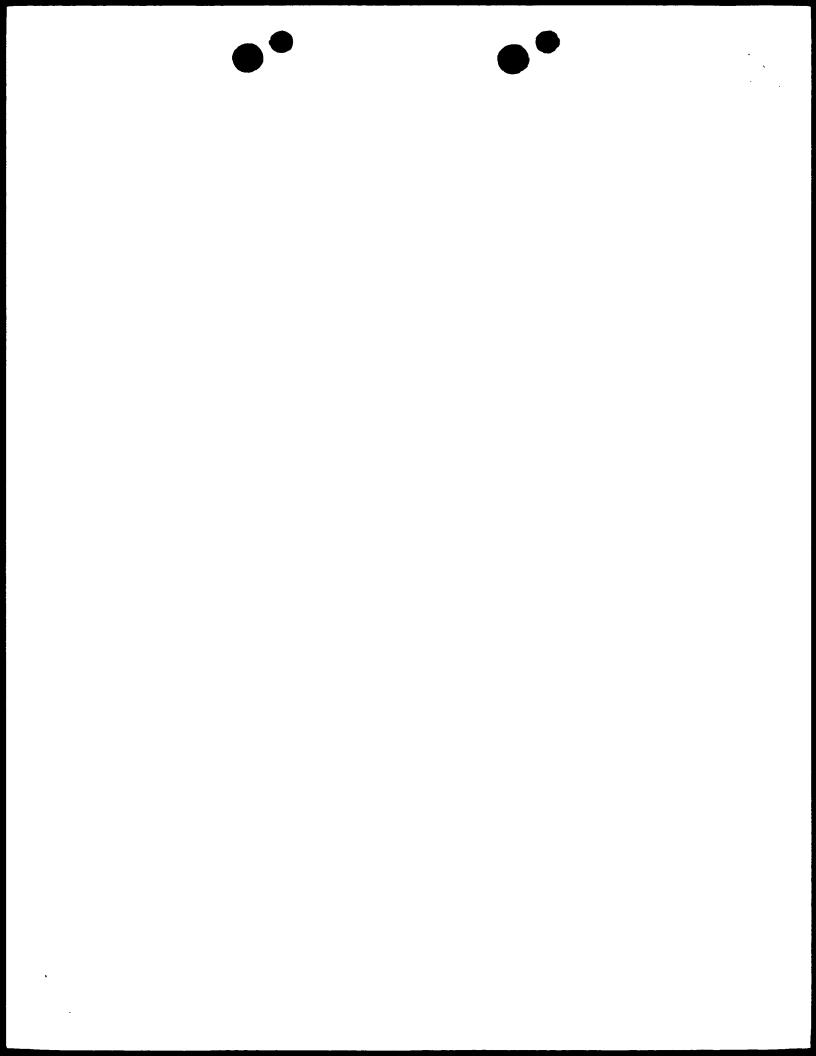Certain published documents (Rule 70.10)

| Application No Patent No | Publication date (day/month/year) | Filing date (day/month/year) | Priority date (valid claim) (day/month/year) |
|---|---|---|---|
| PCT/US98/05441 | 01.10.98 | 19.03.98 | 21.03.97 |

Document PCT/US98/05441 was published after but filed before the priority date of the present application. It does, therefore, not constitute part of the state of the art in the meaning of Rule 64(1)(b) PCT. It will, however become of relevance for the novelty of the claimed subject-matter during regional phase examination at the EPO.
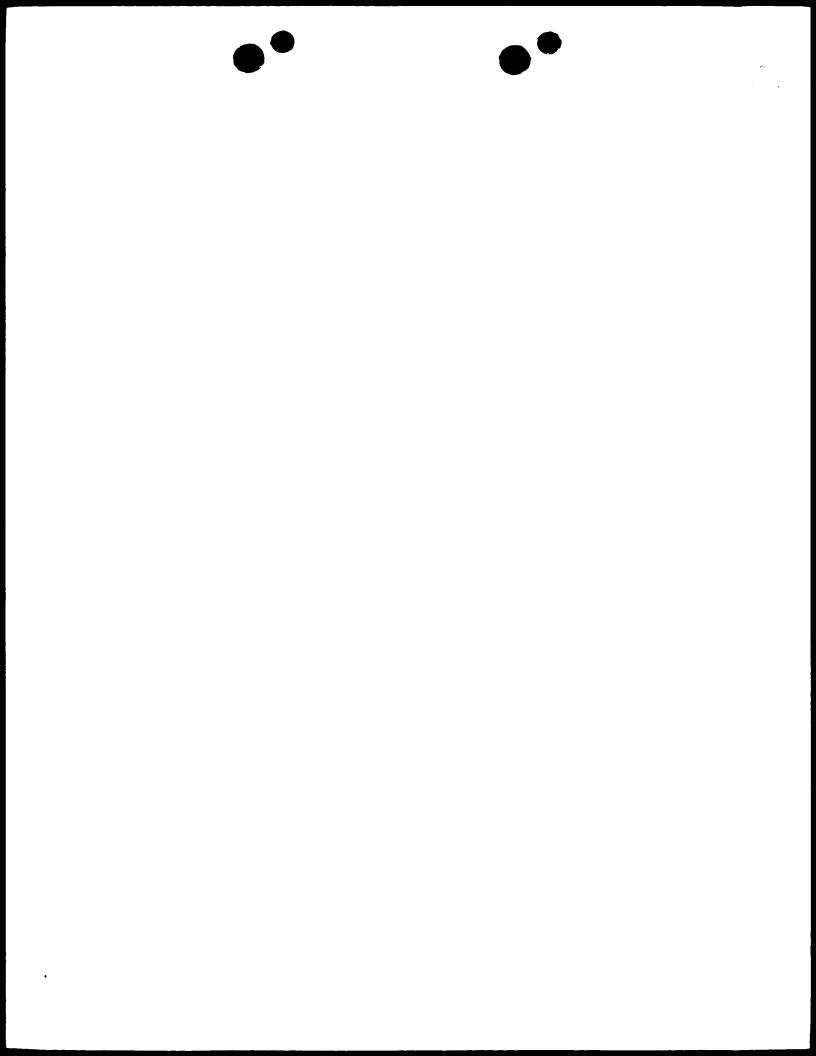
## Re Item VIII
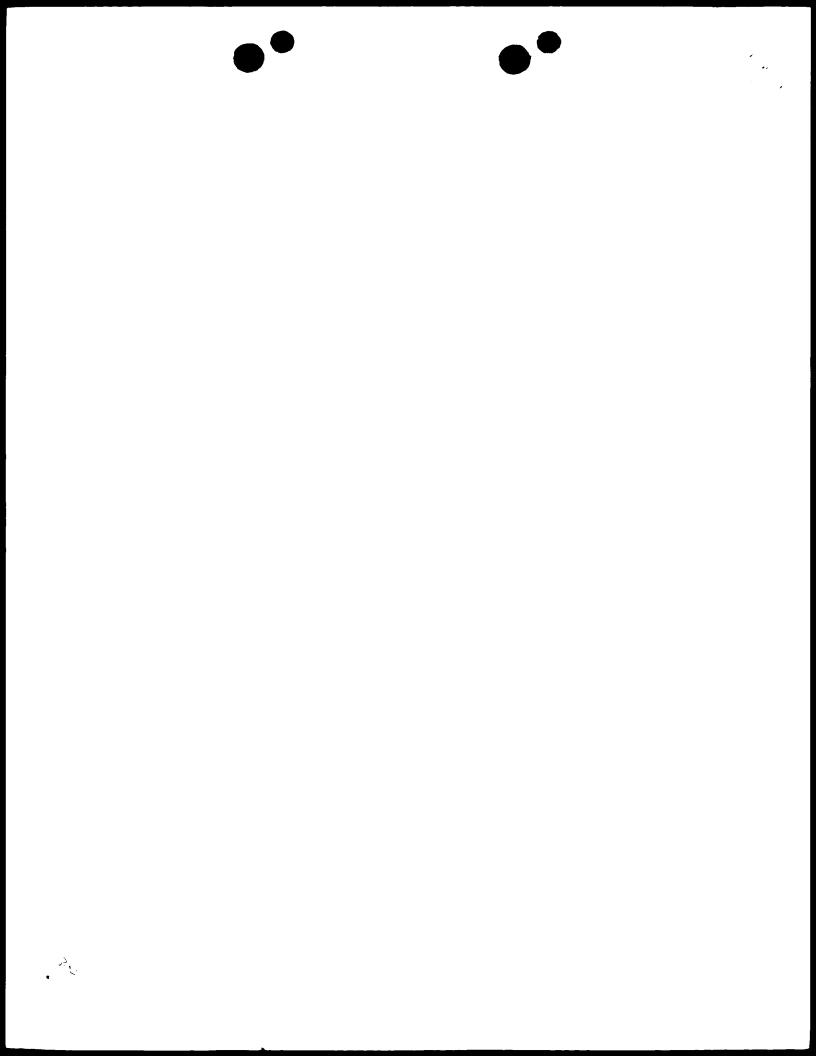## Certain observations on the international application

Clarity (Art. 6 PCT)

i) Claim 22 is unclear due to the relative terms "more immunogenic" and "more stable". The claim attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The claim does not impart any teaching following which the skilled person would be able to produce the required peptide. Immunogenicity and stability depend on the environment in which they are measured, so a modified peptide might be "more" immunogenic when injected in one species and "less" immunogenic in another, the same is true for stability which might depend on several factors such as the solvent, the pH, the temperature etc.

ii) Claim 52 attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The claim does not impart any teaching following which the skilled person would be able to produce the required peptides without undue burden of experimentation. The technical features necessary for achieving the result should be added.

iii) It should be noted that, should the application enter the regional phase at the EPO, disclaimers, such as those present in several of the claims (e.g. 1, 7, 9), aiming at establishing novelty over prior art dealing with the same type of technical problem as the application and relevant for the inventive step of the claimed subject-matter (in the present case D1-D4), are not allowable.

WHAT IS CLAIMED IS

1. A peptide derived from a protein selected from the group consisting of Uroplakin (UP), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Lactadherin (BA-46), Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8 to 10 amino acid residues, of which a second residue from an amino terminal of the peptide and an end residue at a carboxy terminal of the peptide are hydrophobic or hydrophilic natural or non-natural amino acid residues, with the proviso that for PSA, SEQ ID Nos 20 and 24 are excluded, for PSMA, SEQ ID Nos 25, 26, 27, 29 and 30 are excluded and for PAP, SEQ ID Nos 31, 32, 33 and 34 are excluded.

2. The peptide of claim 1, wherein the peptide is derived from Uroplakin.

3. The peptide of claim 2, wherein said Uroplakin is selected from the group consisting of Uroplakin II, Uroplakin Ia, Uroplakin III and Uroplakin Ib.

4. The peptide of claim 3, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:1-19 and 50-64.

5. The peptide of claim 1, wherein the peptide is derived from Prostate specific antigen (PSA) but excluding SEQ ID Nos 20 and 24.

6. The peptide of claim 5, wherein the peptide has a sequence selected from the group consisting of SEQ ID NO's 21-23.

7. The peptide of claim 1, wherein the peptide is derived from Prostate specific membrane antigen (PSMA) but excluding SEQ ID Nos 25, 26, 27, 29 and 30.

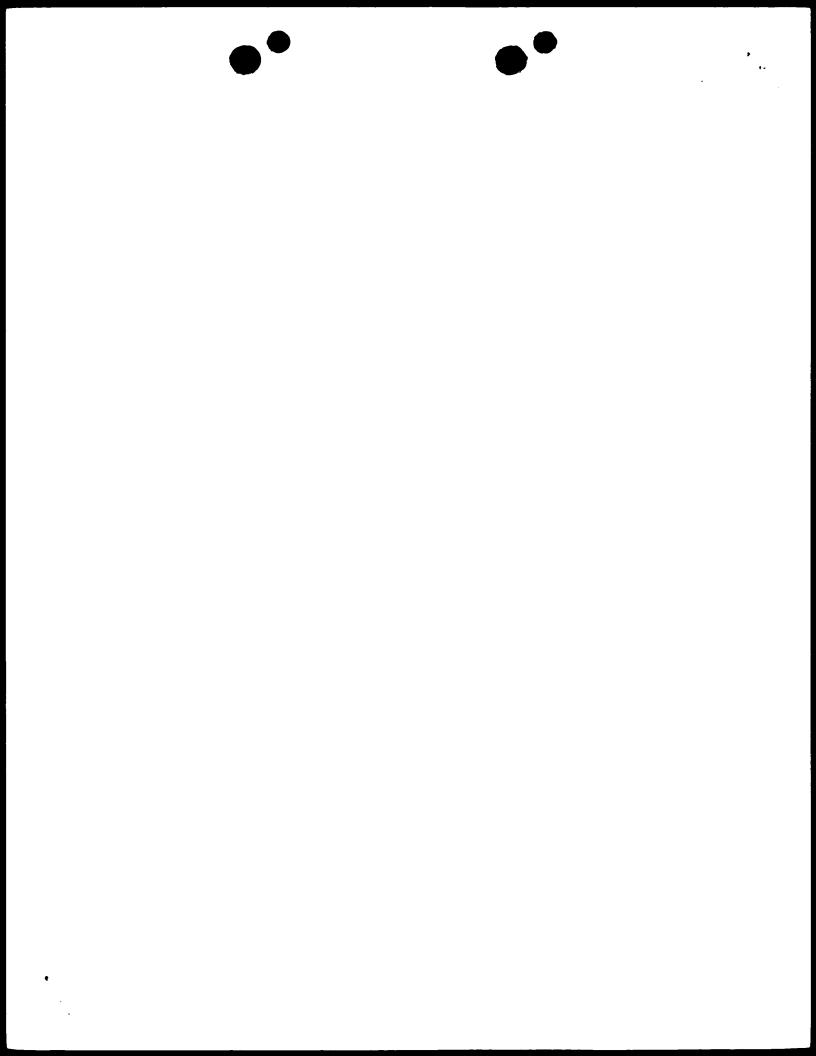8. The peptide of claim 7, wherein the peptide has the sequence identified as SEQ ID NO 28.

9. The peptide of claim 1, wherein the peptide is derived from Prostate acid phosphatase (PAP) but excluding SEQ ID Nos 31, 32, 33 and 34.

10. The peptide of claim 1, wherein the peptide is derived from said Mucin.

11. The peptide of claim 10, wherein the peptide is derived from a non-tandem repeat array of said Mucin.

12. The peptide of claim 10, wherein the peptide is derived from a region selected from the group consisting of a signal peptide, a cytoplasmic domain and an extracellular domain of said Mucin.

13. The peptide of claim 12, wherein the peptide is derived from a non tandem repeat array of said Mucin.

14. The peptide of claim 10, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:42-49.

15. The peptide of claim 1, wherein the peptide is derived from said Lactadherin (BA-46).

16. The peptide of claim 15, wherein the peptide has a sequence selected from the group consisting of SEQ ID NOs:35-41.

17. The peptide of claim 1, wherein the peptide is derived from said Teratocarcinoma-derived growth factor (CRIPTO-1).

18. The peptide of claim 17, wherein the peptide has the sequence selected from the group consisting of SEQ ID Nos. 66-77.

19. The peptide of any of claims 1-18, wherein said peptide is derived from a mammal.

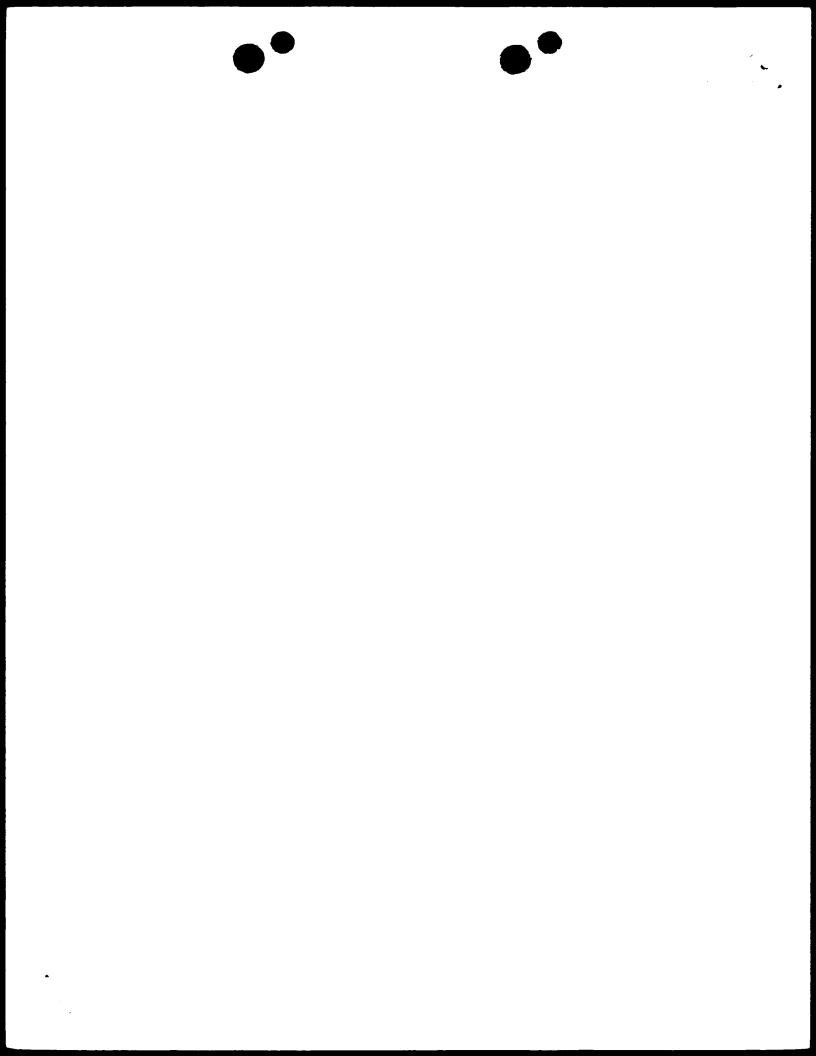20. The peptide of claim 19, wherein the mammal is a humanoid or a rodent.

21. The peptide of any of claims 1-20, wherein said peptide includes at least one non-natural modification.

5   22. The peptide of claim 21, wherein said non-natural modification renders the peptide more immunogenic or more stable than the unmodified peptide.

23. The peptide of claim 21 or 22, wherein said at least one modification is selected from the group consisting of peptoid modification, semipeptoid modification, 10   cyclic peptide modification, N terminus modification, C terminus modification, peptide bond modification, backbone modification and residue modification.

24. A pharmaceutical composition comprising, as an active ingredient, at least one peptide as set forth in any of claims 1-23 and a pharmaceutically acceptable 15   carrier.

25. The pharmaceutical composition of claim 24, wherein said carrier is selected from the group consisting of a proteinaceous carrier to which said at least one tumor associated antigen peptide is linked, an adjuvant, a protein or a 20   recombinant protein and an antigen presenting cell.

26. The pharmaceutical composition of claim 24, wherein the composition contains an amount of said peptide effective to prevent or cure cancer or cancer metastases.

25

27. The pharmaceutical composition of claim 26, wherein said cancer is selected from the group consisting of breast, bladder, prostate, pancreas, ovary, thyroid, colon, stomach and head and neck cancer.

30   28. The pharmaceutical composition of claim 26, wherein said cancer is a carcinoma.

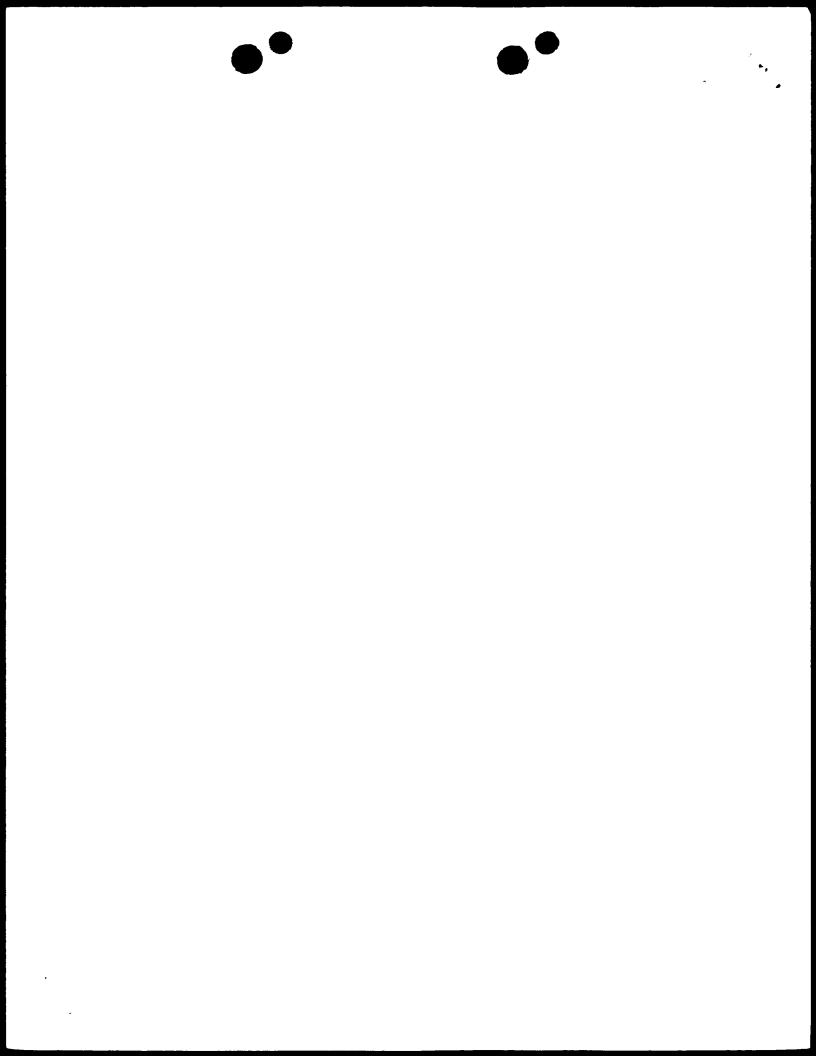29. The pharmaceutical composition of claim 24, wherein the composition is a vaccine.

30. A vaccine composition comprising, as an active ingredient, at least one peptide as set forth in any of claims 1-23 and a suitable carrier.

31. The vaccine composition of claim 30, wherein said carrier is selected from the group consisting of a proteinaceous carrier to which said at least one tumor associated antigen peptide is linked, an adjuvant, a protein or a recombinant protein and an antigen presenting cell.

32. The vaccine composition of claim 30, wherein the composition contains an amount of said peptide effective to prevent or cure cancer or cancer metastases.

33. The vaccine composition of claim 32, wherein said cancer is selected from the group consisting of breast, bladder, prostate, pancreas, ovary, thyroid, colon, stomach and head and neck cancer.

34. The vaccine composition of claim 32, wherein said cancer is a carcinoma.

35. A method of prevention or cure of a cancer or of metastases thereof comprising the step of administering to a patient an effective amount of the pharmaceutical composition of any of claims 24-29.

36. A method of prevention or cure of a cancer or of metastases thereof comprising the step of vaccinating a patient with an effective amount of the vaccine composition of any of claims 30-34.

37. A polynucleotide encoding at least one peptide according to any of claims 1-23.

38. A polynucleotide encoding at least one peptide selected from the group consisting of SEQ ID Nos. 1-19, 21-23, 28, 35-64 and 66-77.

39. The polynucleotide of claim 37 or 38, wherein the polynucleotide forms a part of a longer polynucleotide designed to encode a fused protein product from which said at least one peptide is cleavable by a protease.
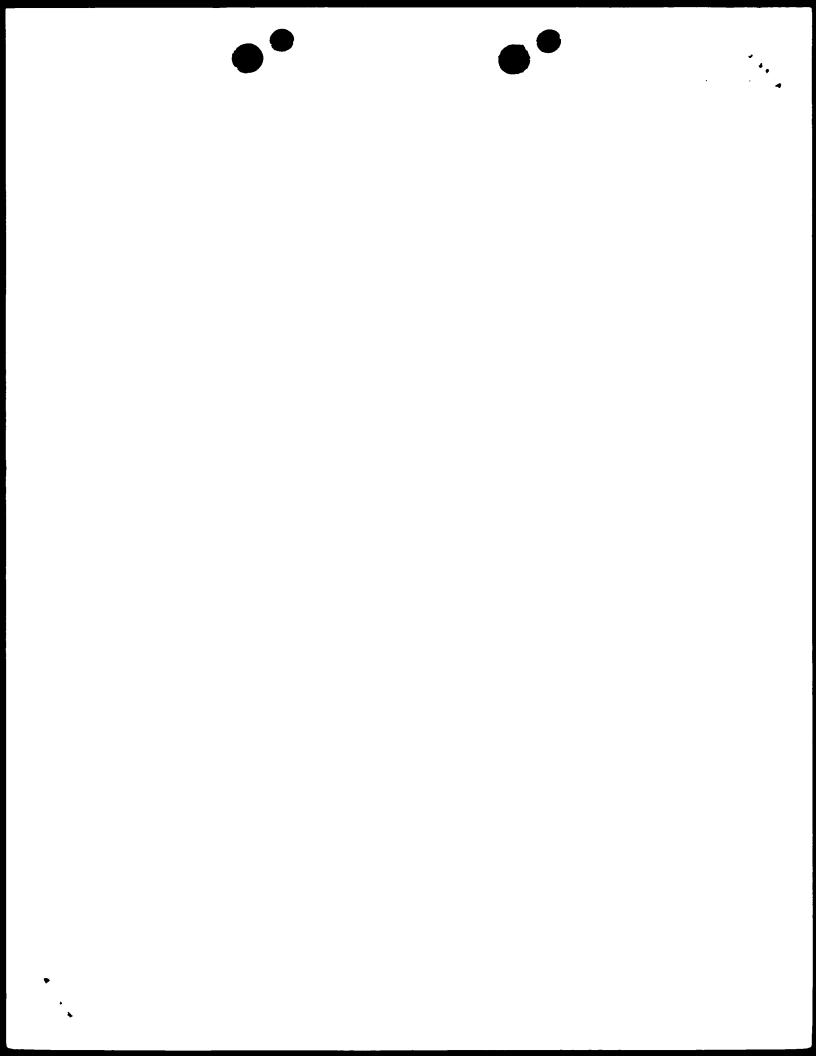
40.     A pharmaceutical composition comprising, as an active ingredient, at least one polynucleotide as set forth in any claims 37-39 and a pharmaceutically acceptable carrier.

5       41.     A cellular vaccine composition comprising an antigen presenting cell presenting at least one peptide of any of claims 1-23.

42.     The cellular vaccine composition of claim 41, wherein said antigen presenting cell is selected from the group consisting of a dendritic cell, a macrophage, a B
10      cell and a fibroblast.

43.     The cellular vaccine composition of claim 41, wherein said antigen presenting cell is caused to present said at least one tumor associated antigen peptide by a method selected from the group consisting of:
15      a)      genetically modifying said antigen presenting cell with at least one polynucleotide encoding said at least one tumor associated antigen peptide such that said peptide or at least one longer polypeptide including said peptide will be expressed;
        b)      loading said antigen presenting cell with at least one polynucleotide
20              encoding said at least one tumor associated antigen peptide;
        c)      loading said antigen presenting cell with said at least one tumor associated antigen peptide; and
        d)      loading said antigen presenting cell with at least one longer polypeptide including said at least one tumor associated antigen peptide
25

44.     The peptide of claim 1, wherein the second residue and the end residue are neutral, hydrophobic and aliphatic.

45.     The pharmaceutical composition of any of claims 24-29 or 40 also comprising
30      a helper peptide.

46.     The pharmaceutical composition of claim 45, wherein the helper peptide has a T helper epitope.

47.    The vaccine composition of any of claims 30-34 also comprising a helper peptide.

48.    The vaccine composition of claim 47, wherein the helper peptide has a T helper epitope.

49.    Use of at least one peptide of claims 1-23 in the manufacture of a medicament.

50.    The at least one peptide of claims 1-23 for use as a medicament.

51.    The use of claim 49 or 50, wherein the medicament is effective to prevent or cure a cancer or cancer metastases.

52.    A peptide derived from a protein selected from the group consisting of Uroplakin (UP), Lactadherin (BA-46), Prostate specific antigen (PSA), Prostate specific membrane antigen (PSMA), Prostate acid phosphatase (PAP), Mucin (MUC1) and Teratocarcinoma-derived growth factor (CRIPTO-1), the peptide comprising 8-10 amino acid residues selected so as to promote effective binding to a MHC class 1 type molecule so as to elicit a CTL response.

*P Camywp/048*

AMENDED SHEET

(54) Title: COMPUTERIZED GAMING SYSTEM

(57) Abstract

A computerized gaming system including a plurality of remotely located multiple or single win game machine terminals (10) and a remotely located central processing station (32). Each of the game machine terminals is computerized and enables a player to attempt to win a prize by matching player selected indicia (24) with random indicia (22) generated by the central processing station. The game machine terminal pays off prizes in accordance with a predetermined prize schedule (28) and the number of matches between the player selected indicia and the randomly generated indicia. Even if no matches are made between the player selected indicia and the randomly generated indicia, a consolation prize will be awarded if any indicia repeats in the randomly generated indicia a predetermined number of times. If the prize to be awarded is less than a predetermined value, the game machine terminal immediately pays out the prize to the player. If the prize to be awarded is greater than a predetermined value, visual and audible alarms (66) associated with the game machine terminal go off, indicating that the player has won a grand prize. In this instance, a validation ticket indicating the identification code of the game machine terminal producing the winning prize, the time and date on which the game was played, a validation code and the prize amount won is issued to a casino employee at the central processing station. The casino employee then hand carries the validation ticket to the game machine terminal and presents the ticket to the player after the employee has determined that the game machine terminal has not been tampered with. The player is then free to cash in the ticket at cashier's station in the casino.

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## COMPUTERIZED GAMING SYSTEM

BACKGROUND OF THE INVENTION

The present invention is directed towards a
novel method and apparatus for playing a game of
chance, more particularly for playing a modified slot
5      machine type game.  In the past, slot machines were
primarily electro-mechanical devices which operate
independently of each other.  While there are several
variations on the standard slot machine, they generally
include three symbol wheels containing a plurality of
10     picture symbols (such as cherries and oranges) which
are rotated responsive to the actuation of a switch arm
coupled to the slot machine.  The symbol wheels stop at
"random" locations to display three picture symbols.
If at least two of the symbols match, the slot machine
15     pays out a predetermined prize which varies as a function
of the amount of money wagered and the odds of matching
the symbols displayed.

While the standard slot machine has proven to
be very popular, it has several drawbacks.  Due to its
20     electro-mechanical nature, its frequency of repair
record is relatively high.  Additionally, since each
machine is independent of the remaining machines, the
casino does not have continuous access to information
regarding the total amount wagered and total amount

paid out by the various machines at the casino.
Finally, while the standard slot machine format has
maintained the public interest for many years, casinos
are continually looking for improved variations on this
5    format to increase the interest of the casino patrons.


BRIEF DESCRIPTION OF THE INVENTION

           In order to overcome the foregoing drawbacks,
the present invention is directed towards a computerized
gaming system including a plurality of remote game
10   machine terminals (each in the form of a modified slot
machine) and a central computer.  Each of the game
machine terminals is computerized and contains a minimum
number of mechanical parts.  Additionally, each of the
remote game machine terminals continuously communicates
15   with the central computer in a manner which enables the
central computer to keep a continually updated record
of the total amount of money wagered and the total
amount of money paid out by each of the game machine
terminals.  Finally, the present invention provides an
20   improved slot machine format by permitting the player
to select random indicia to be matched, providing the
player with a sense of participation.

           In the preferred embodiment of the invention,
each game machine terminal takes the form of a modified
25   slot machine including six electronic numerical displays
and a keyboard which enables the player to select any
one of the digits 0-9.  Indicia other than numbers
such as card symbols, bells, fruits, etc. could also be
used.  The object of the game is to match the player
30   selected number with a randomly-generated number which
is displayed on the numerical displays responsive to
actuation of a switch arm of the game machine terminal.
The game machine terminal pays off prizes in accordance

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with a predetermined prize schedule and the number of
matches between the player selected number of indicia
and the randomly-generated number of indicia.  Even if
no matches are made between the player selected number

5    and the randomly-generated number, a consolation prize
will be awarded if any digit (e.g., 7) repeats in the
randomly-generated number a predetermined number of
times (e.g., 4 times).  If the prize to be awarded is
less than a predetermined value, the game machine

10   terminal immediately pays out the prize to the player.
If the prize to be awarded is greater than the pre-
determined value, visual and audible alarms associated
with the game machine terminal go off, indicating that
the player has won a grand prize.  In this instance, a

15   validation ticket indicating the identification code of
the game machine terminal producing the winning prize,
the time and date on which the game was played, a validation
code and the prize amount won is issued to a casino employee
by the central computer.  This will normally be done in an

20   office area of the casino.  The casino employee then
hand carries the validation ticket to the game machine
terminal and presents the ticket to the player after
the employee has determined that the game machine
terminal has not been tampered with.  The player is

25   then free to cash in the ticket at a cashier's station
in the casino.


## BRIEF DESCRIPTION OF THE DRAWINGS

For the purpose of illustrating the invention,
there are shown in the drawings several embodiments

30   which are presently preferred; it being understood,
however, that this invention is not limited to the
precise arrangements and instrumentalities shown.

Figure 1 is a perspective view of the housing of a game machine terminal which forms part of the computerized gaming system of the present invention.

Figure 2 is a block diagram of the computer-
5    ized gaming system of the present invention.

Figure 3 is a block diagram of the structure of the game machine terminal.

Figure 4 is a block diagram of the structure of the central computer which forms part of the computer-
10   ized gaming system of the present invention.

Figures 5a and 5b are a flow diagram of a program controlling the operation of each game machine terminal of the present invention.

Figure 6 is a flow diagram of the program
15   controlling the central computer of the present inven-
tion.

Figure 7 is a schematic diagram of a second embodiment of a game machine terminal which may be utilized in connection with the present invention.

20   DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now to the drawings wherein like numerals indicate like elements, there is shown in Figure 1 a game machine terminal constructed in ac-cordance with the principles of the present invention
25   and designated generally as 10. Game machine terminal 10 preferably takes the form of a modified slot machine including a housing 12, a switch actuating arm 14, a display panel 16, a coin receiving slot 18 and a coin dispensing slot 20. Display panel 16 includes a digital
30   display 22 and a keyboard 24. The object of game machine terminal 10 is to permit a player to wager a desired amount and attempt to win a prize by matching a player selected number with a psuedo-random number generated in response to the actuation of arm 14.

35

- 5 -

A player initiates the game by depressing one
of the push buttons of keyboard 24, so as to select the
desired number (e.g., 7), and depositing a coin or
token in coin receiving slot 18. The player then pulls
5   switch actuating arm 14 to initiate the generation of
the psuedo-random number on display 22. Upon actuation
of switch arm 14, the individual digits of digital
display 22 begin to incrementally advance at variable
speeds and, after a predetermined time period, stop
10  sequentially to display a psuedo-random number (473777
in the example shown). Game machine terminal 10 then
determines the number of matches between the selected
number (7) and the psuedo-random number (473777) and
computes a prize amount determined by the number of
15  matches and the amount wagered. If the prize amount is
below a predetermined value, for example $250.00, game
machine terminal 10 immediately pays out the prize by
depositing coins or tokens in coin receiving tray 26.
If the prize amount is greater than the predetermined
20  value, lights behind enunciator display 28 begin flashing
and an audible signal (such as a bell or a siren) is generated
to indicate a grand prize winner. As will be explained in
greater detail below, a validation ticket is then hand
carried by an employee of the operator of gaming system 30
25  (normally a casino) to the winning machine and presented to
the player after the employee determines that the machine
had not been tampered with. The player may then cash the
validation ticket in with the casino cashier.

As indicated by the prize structure set forth
30  on enumerator display 28 of Figure 1, game machine
terminal 10 is capable of playing two games. The first
game compares the number selected by the player with

the random number displayed on digital display 22 and determines the amount won as a function of the number of matches and the amount wagered.  In the example illustrated in Figure 1, game machine terminal 10 would

5    pay out $25.00 if a $1.00 token was deposited in coin receiving slot 18.  The machine terminal 10 can be set to accept coins of various denominations of, for example, 5¢, 10¢, 25¢, 50¢ or $1.00 by interchanging the coin acceptor within the machine.  In a second game played

10   by game machine terminal 10, a comparison is made between the individual digits displayed in digital display 22 and a consolation prize is awarded if any number occurs at least, for example, four times in the pseudo-random number.  In the example shown, if any

15   number other than number 7 had been selected, the player would be entitled to a payoff of $2.00 for a $1.00 wager based upon the consolation prize payoff schedule illustrated on display 28.

        While Figure 1 discloses a single game machine

20   terminal 10, the present invention contemplates a computerized gaming system 30 (see Figure 2) including a plurality of remote game machine terminals 10a, 10b, 10c, 10d ... 10n which communicate with a central computer 32 via a system bus 34 or other communication

25   network.  Central computer 32 generates the psuedo-random numbers displayed by each game machine terminal 10 and maintains a complete record of each game played in- cluding the total amount wagered, the coin denomina- tion of each machine, and the total amount paid out by

30   each machine terminal 10.  Additionally, central com- puter 32 generates a validation ticket whenever a prize amount above the predetermined value is won by a player of any of the game machine terminals 10.  The valida- tion ticket is issued to an employee of the casino and

indicates both the terminal identification code of the
winning game machine terminal 10 and the amount of the
prize a validation code together with any other desired
information.   The validation ticket is hand carried by
5   the casino employee to the appropriate game machine terminal
and handed to the player of that machine after the casino
employee determines that the machine had not been tampered
with.

The manner in which computerized gaming system
10   30 carries out the foregoing functions will now be described
with reference to Figures 3-6.   Figure 3 is a block diagram
of a single game machine terminal 10.   Each of the game
machine terminals 10a-10n are identical in structure.   Ac-
cordingly, the structure and operation of only a single
15   such terminal will be described.

The heart of game machine terminal 10 is a
central processing unit (CPU) 36 which controls the
operation of game machine terminal 10 in accordance
with a program stored in ROM memory 38.   CPU 36 may be
20   any commercially available microprocessor, by way of
example, an INTEL 8080.   ROM memory 38 may be any
suitable read-only memory.   The program stored in
memory 38 causes CPU 36 to step game machine terminal
10 through each of the steps set forth in the flow
25   diagram of Figures 5a and 5b, and described in detail
below.   In addition to storing the program, memory 38
stores a multi-digit terminal identification code which
is unique to and identifies both the particular game
machine terminal 10 with which memory 38 is associated
30   and the denomination of coil that coil acceptor 56 is
set for.   By way of example, terminal 10a, a $1.00
machine, may be identified by the terminal number
1000000 while game terminal 10b, a 5¢ machine, could be
identified by the terminal number 5000001.

      In addition to ROM memory 38, each terminal 10 includes a random access memory (RAM) 40. Random access memory 40 may be any commercially available RAM and serves to temporarily store information received

5    from central computer 32. As will be shown below, each game terminal 10 communicates with the central computer 32 by an appropriate communications link such as system bus 34. When transmitting information between the various game machine terminals 10 and central computer

10   32, it is preferable to encode the information transmitted on the communication system to prevent tampering with the system. To this end, game machine terminal 10 includes a data encryption unit 42 which encodes the data transmitted from the game machine terminal 10 to

15   central computer 32 and decodes the data received by game machine terminal 10 from central computer 32. While any data encryption unit may be utilized, there are several commercially available microprocessor peripheral units (e.g. INTEL Part No. 8294) designed to

20   encode and decode 64 bit blocks of data using the algorithm specified in the Federal Information Processing Data Encryption Standard. Such a data encryption unit operates on 64 bit text words using a 56 bit user specified key to produce 64 bit cipher words.

25   The algorithm specified by the Federal Information Processing Data Encryption Standard is permanently contained in the data encryption unit while the user specified key is stored in memory 38. As such, the 56 bit key may be changed at any time by the operator of

30   the computerized gaming system 30.

      The operation of display panel 16 is controlled by keyboard section 44 which includes digital display 22, keyboard 24, a lever key 50 and a keyboard and display interface 52. Digital display 22 (Figure 1)

35   includes a plurality (six in the example shown) of

individual displays which are preferably seven segment
LED or LCD displays.  As will be shown below, these
displays are stepped through their various outputs
(i.e., 0, 1, 2 ... 9) at a high rate of speed responsive
5  to the actuation of switch actuating arm 14.  As a
result, the individual displays give the appearance
that they are "rotating" in a manner similar to the
pictorial display wheels of a standard slot machine.
While each individual display of digital display 22 is
10  preferably a numerical display, displays which utilize
any type of indicia (e.g., alphabetical or pictorial)
may also be used.  Additionally, standard electro-
mechanical displays may be substituted for the electronic
displays illustrated.

15       Still referring to Figure 1, keyboard 24
includes a plurality of push button switches which are
preferably back lighted interlock switches.  The switch
last depressed represents the number selected by the
player and is back lit to the exclusion of the remaining
20  switches.  While each switch of keyboard 24 is shown as
representing a different digit, they can also represent
letters or pictorial displays in accordance with the
type of display used in digital display 22.  While push
button switches are illustrated, any other appropriate
25  selector switch may be used.

       Lever key 50 is preferably a single pole,
double throw snap switch which is connected to switch
actuating arm 14 and closes whenever switch actuating
arm 14 is pulled.  When key lever 50 is actuated, it
30  generates a game initiator signal which causes the
information on keyboard 24 (i.e., the number selected)
to be applied to keyboard and display interface 52
which generates a digital code representative of this
information and places it on data bus 54.  Interface 52
35  also causes the "rotation" of the individual digits of

digital display responsive signals generated by CPU 36.
See below.

      The coin receiving and dispensing operations
of game machine terminal 10 are controlled by coin
5    receiving and dispensing section 46. A coin acceptor
56 is located behind coin receiving slot 18 (Figure 1)
and receives a specific denomination of coins (e.g.,
$1.00) deposited by the player. Coin acceptor 56
verifies the receipt of a proper denomination coin or
10   token and causes the count in coin count meter 60 to
increase by one each time a non-bogus coin or token is
deposited in slot 18. The count in coin count meter 60
thereby represents the amount wagered by the player.
The information in coin count meter 60 is applied to
15   data bus 54 in the form of a coded signal by peripheral
interfact 64.

      Coin dispenser 58 is located behind coin
dispensing slot 20 and dispenses coins or tokens of the
same denomination that coin acceptor 56 has been de-
20   signed to accept into coin receiving tray 26 responsive
to instructions received from CPU 36 via data bus 54.
This information is generated in the form of digital
signals on bus 54 and applied to coin dispenser 58 by
peripheral interface 64. Coin count meter 62 counts
25   the number of coins generated by coin dispenser 58 and
thereby insures that the appropriate number of coins
are dispensed.

      Visual and audible enunciator 66 is located
behind the translucent face of enunciator display 28
30   and includes audible and visual signal generators which
provide an indication that a grand prize (i.e., one
greater than the predetermined amount) has been won.
Enunciator 66 is enabled by CPU 36 (via interface 64)
whenever a grand prize has been won.

35

Coin acceptor 56 and coin dispenser 58 are commercially available devices and may be obtained from various manufacturers of vending machines such as Rowe, Inc., Coin Acceptors, Inc., and Bally Manufacturing
5    Company. Coin counter meters 60 and 62 are preferably electro-mechanical meters which may be obtained from Durant Company or Hecon, Inc. Peripheral interface 64 is similarly a commercially available device which may be obtained from INTEL under their designation Part No.
10   M8255A.

As noted above, game machine terminal 10 operates in cooperation with central computer 32. Information is transmitted between game machine terminal 10 and central computer 32 via a data bus or other
15   appropriate transmission system such as a modem. To this end, terminal 10 includes a communication inter-face 68 which receives the encoded data (from data encryption unit 42) which is located on data bus 54 and converts this information into a form which may be
20   efficiently transmitted on the transmission system connecting terminal 10 with central computer 32. One suitable communication interface is available from INTEL under the designation Part No. M8251. If the information transmitted between terminal 10 and com-
25   puter 32 is transmitted via a modem, the output of communication interface 68 is applied to a standard RS232C connector which applies the information to the phone line. If the information is transmitted via a standard system bus (see Figure 2), the output of
30   interface 68 is applied directly to the data bus.

The structure of central computer 32 is illustrated in Figure 4. As shown therein, central computer 32 includes a communication interference 70 and a

data encryption unit 74 which are identical in structure
and function to interface 68 and encryption unit 42,
respectively. Central computer 32 also includes a CPU
76 which is preferably more sophisticated than CPU 36
5    and is capable of responding to a high order language
program (e.g., BASIC or FORTRAN) stored in floppy disc
memory 78. As will be described in greater detail
below with reference to Figure 6, CPU 76 generates a
psuedo-random number responsive to receipt of the
10   information generated by the game machine terminal 10
and compares the psuedo-random number to the number
selected by the player. CPU 76 then determines the
player's winnings in accordance with the prize structure
illustrated on enunciator display 28 and generates a
15   two digit win code (other win codes may be used) indica-
tive of the prize amount. The psuedo-random number,
terminal identification code, number selected and win
code are stored, along with the time and date indicated
by calendar clock 80, in memory 78. In this manner,
20   floppy disc memory 78 maintains a continuous history of
the operation of gaming system 30 including the total
amount wagered and the amount paid out.

In addition to storing the information in
memory 78, CPU 76 returns information concerning the
25   psuedo-random number generated, the terminal identifica-
tion code, the player selected number and the win code
to the game machine terminal 10 after encryption by
data encryption unit 74. This information is used by
game machine 10 to provide a visual indication of the
30   psuedo-random number generated and to pay the prize
amount in a manner described below. In the event that
the prize amount is greater than a predetemined number
of coins, CPU 76 causes line printer 82 to print out a
validation ticket containing information regarding the

identification code of the terminal 10 being played,
the time and date at which the game was played, the
amount wagered, a validation code and the amount won.
This ticket is issued to a casino employee for verifica-
5    tion at the terminal 10 in the manner described above.
This ticket may also include a code identifying the
particular gaming system 30. While central computer 32
preferably issues a validation ticket, the present
invention encompasses the use of any type of informa-
10   tion record medium which will inform the casino employee
of the foregoing information.

In the preferred embodiment, central computer
32 also includes an on-line keyboard/display terminal
84 which permits the operator of gaming system 10 to
15   withdraw any desired information from memory 78 in-
cluding the total amount paid out during that time
period. Each of the elements of central computer 32
are standard computer elements and may be purchased
from several large computer firms.

20   The operation of gaming system 30 will now be
described with reference to the flow diagrams of Figures
5 and 6. As noted above, the player initiates the game
by depressing one of the push button switches of keyboard
24, depositing a coin or token in slot 18 (thereby
25   incrementing the count in meter 60) and pulling switch
actuating arm 14. See blocks 86 and 88 of Figure 5A.
When switch actuating arm 14 is pulled, lever key 50 is
closed causing a game initiation or interrupt signal to be
generated and applied to data bus 54 via interface 52.
30   CPU 36 senses this signal and, in accordance with the
program stored in memory 38, requests data from keyboard
24 identifying the number selected by the player. This
information is transmitted via keyboard and display
interface 52 and is stored in a register of CPU 36.
35   See block 90. Thereafter, CPU 36 applies a digital

signal indicating that the game has been initiated to
data bus 54. This information is stored in memory 40.
Immediately thereafter, CPU 36 applies information
concerning the player selected number (as determined by
5   keyboard 24), the identification code of terminal 10
(stored in memory 38) and the user selected key (also
stored in memory 10) to data encryption unit 42. Data
encryption unit 42 codes this information in accordance
with the specified key and places the coded information
10   on data bus 54. Thereafter, CPU 36 causes the encoded
information to be transmitted to central computer 32
via communication interface 68. See block 92. The
transmitted signal will be referred to herein as a
request signal. The central computer accepts and acts
15   on each request in the queing order in which the request
was received.

      After transmitting the encoded request signal
to computer 32, CPU 36 instructs interface 52 to begin
incrementing each of the individual displays of digital
20   display 22. As indicated in blocks 94-104, a sub-
routine in the program stored in memory 38 causes CPU
36 to generate signals which cause each of the indivi-
dual displays of digital display 22 to increment at
different speeds. By way of example, the number
25   displayed by the first display 22a will be incremented
(i.e., from 0 to 1, from 1 to 2, etc.) every 75 milli-
seconds while the second display 22b will be incremented
once every 100 milliseconds. By varying the rate at
which each of the individual displays of digital display
30   22 increment between 75 and 175 milliseconds, each of
the displays will give the appearance of "rotating".
CPU 36 continues to generate signals which cause the
individual displays of digital display 22 to increment
at their respective rate until the psuedo-random number
35   is returned from central computer 32.

After initiating the "rotation" of each of
the displays of digital display 22, CPU 36 waits 100
milliseconds to determine if it has received return
data from computer 32. See block 106. At the end of
5    this time interval, CPU 36 determines whether or not
data, in the form of a response signal, has been re-
ceived from central computer 32. If the response
signal has been received, the game is completed in the
standard manner described below. If a response signal
10   has not been received at the end of the 100 millisecond
delay period, CPU 36 causes coin dispenser 58 to
return the coin or token wagered, disables display 22
and posts an out-of-order light which may be located on
the enunciator display 28. See blocks 110, 112, and
15   114. In this condition, game machine terminal 10 is
out of operation and must be repaired by a casino
employee before again being placed in use.

        Referring now to Figures 4 and 6, the operation
of central computer 32 will now be described. The
20   request signal containing the terminal identification
code and player selected number transmitted by terminal
10 are received by communication interface 70 and
placed on data bus 72. The encoded information is now
decoded by data encryption unit 74 and applied to CPU
25   76. Upon receipt of this information, CPU 76, under
program control of memory 78, generates the psuedo-
random number. See block 118. By way of example, the
psuedo-random number may be generated utilizing an
intrinsic function command (such as RND in the language
30   BASIC) and using a random feed from a free-running
incrementing storage register (RND (N)) so as to gen-
erate a multi-digit random number. This number is then
rounded off to the required number of digits (6 in the
embodiment illustrated in Figure 1) and is stored in
35   one of the registers of CPU 76. CPU 76 compares the

pseudo-random number with the player selected number and generates a win code indicative of the prize won. See block 120. One suitable two digit win code is illustrated in the following table:

5

<div align="center">

TABLE I

</div>

| | | |
|---|---|---|
| No match | 00 | No payout, end game |
| 1 match | 01 | payout 1 coin |
| 2 match | 02 | payout 2 coins |
| 3 match | 03 | payout 10 coins |
| 4 match | 04 | payout 25 coins |
| 5 match | 10 | activate enunciator |
| 6 match | 20 | activate enunciator |
| any four of a kind | 05 | payout 2 coins |
| any five of a kind | 06 | payout 50 coins |
| any six of a kind | 30 | activate enunciator |

The win code, along with a psuedo-random number, is stored in one of the buffers of CPU 76. At this point, CPU 76 determines whether the amount won is over the predetermined value (e.g., 250 coins). See block 122. If the amount won is less than 250 coins, the psuedo-random number generated, the terminal identification code, the player selected number and the win code are encoded by data encryption unit 74 and transmitted back to terminal 10. See blocks 124, 126. If the amount won was over 250 coins, CPU 76 causes line printer 82 to print out a voucher including all of the information described above. See block 128. Thereafter, the psuedo-random number generated, the terminal identification code, the player selected number and the win code are encoded by data encryption unit 74 and retransmitted to terminal 10 in the form of an encoded response signal. See blocks 124 and 126.

Finally, information concerning play and win data is
stored in floppy disc memory 78. See block 129.

      Returning now to Figures 3 and 5, the manner
in which game machine terminal 10 responds to the
5    response signal transmitted by central computer 32 will
now be described. The response signal transmitted by
central computer 32 is applied to data bus 54 of termi-
nal 10 via communication interface 68. All game machine
terminals (10) monitor system bus 34 for response
10   signals during a "response signal" mode and accept only
that data train that corresponds to its own address
(identification number) and acknowledges acceptance to
central computer 32 through a "handshake" signal. The
encoded information is decoded by data encryption unit
15   42 and placed in RAM memory 40. CPU 36 checks the
received response signal to determine if it contains
the identification code of the terminal 10 with which
CPU 36 is associated. If not, the response signal
information is erased from memory 40 and CPU 36 awaits
20   receipt of a new response signal. If the response
signal contains the identification code associated with
CPU 36, CPU 36 responds thereto as follows. Upon
receipt of the proper response signal, CPU 36 dampens
the rate of incrementation of each of the displays of
25   digital display 22 to give the appearance that the displays
are rotating at an exponentially decreasing speed. After
approximately 300 milliseconds, CPU 36 causes the
first display 22a to stop incrementing at the number
corresponding to the first digit of the psuedo-random
30   number generated in memory 40. See block
300 milliseconds later, CPU 36 causes the second
display 22b to stop incrementing at the number corres-
ponding to the second digit of the psuedo-random number
(e.g., 7). See block 132. This information is repeated
35   for the third, fourth, fifth and sixth displays (blocks
134-140) so that each of the six digits of the psuedo-
random number is displayed on digital display 22.

CPU 36 then compares the psuedo-random number stored in memory 40 with the player selected number to determine which digit positions present a match between the preselected number and the psuedo-random number.

5 See block 142. CPU 36 then causes those displays which match with the preselected number (e.g., displays 22b, 22d, 22e and 22f in the example shown) to indicate a match between these numbers and the preselected numbers. See block 144.

10 After initiating the flashing of the appropriate displays, CPU 36 withdraws the win code from memory 40 and determines whether or not the win code indicates that the player has won a prize. If the win code indicates that no prize has been won (e.g., a code of

15 00), the game is ended and game machine terminal 10 is ready to accept a new wager. See decision blocks 146. If the win code indicates that the player has won, CPU 36 then determines if the amount won is over the pre-determined value (e.g., 250). See decision block 148.

20 If the amount won is less than the predetermined value, CPU 36 places a digital signal on data bus 54 which causes coin dispenser 58 to dispense coins until coin count meter 62 indicates that the appropriate number of coins have been dispensed. See block 150. At this

25 point, the game is completed and terminal 10 is again ready to accept a new wager.

If the prize amount is greater than the predetermined value, CPU 36 enables visual and audible enunciator 66 thereby indicating a grand prize. See

30 block 152. At this point, the program in memory 38 halts and the machine is effectively disabled until it receives a clear code from computer 32. See block 154. The clear code will not be generated by the computer until the casino employee determines that the machine

35 has not been tampered with and enters the clear code on

keyboard-display terminal 84 (see Figure 4). See block
156. When machine terminal 10 receives the clear code
from computer 32, CPU 36 rests terminal 10 and the
terminal is again ready to receive another wager. See
5   block 158.

In the foregoing embodiment, only a single
prize structure was utilized in connection with each
game machine terminal 10. In accordance with the second
embodiment to the present invention, the prize structure
10  of the game may be changed randomly or according to the
time of day. As shown schematically in Figure 7, enunciator
display 28 may be divided into two halves 22a and 22b
illustrating the respective prize structures A and B.
During those portions of the day in which prize structure
15  A is used, the portion 22a of enunciator display 22
will be back lit. Similarly, during those portions of
the day in which prize structure B is used, portion 22b
is back lit. The change in prize structure may be
controlled by an internal clock in game machine terminal
20  10 or by signals received from central computer 32
which has its own timing clock 80. In either case,
central computer 32 keeps track of which machines are
under the control of which prize structure. The advantage
of changing the prize structure in the foregoing manner
25  is to encourage greater play during off peak hours or to
add interest at random time during the day.

In another modification of applicant's invention,
central computer 32 may be programmed to generate a random
bonus win at machine terminal 10 irrespective of the psuedo-
30  random number generated and the player selected number. By
way of example, the bonus win may be generated randomly or
the central computer 32 may be programmed to count the number
of games played by gaming system 30 and generate a bonus
win responsive to each thousandth game played. Such
35  a "random" bonus adds excitement to the game played by

each machine terminal 10. Finally, while game machine
terminal 10 has been described in connection with an
embodiment that can receive only one coin or token, one
skilled in the art could easily modify the program

5     illustrated in Figures 5 and 6 to enable each machine
terminal 10 to accept a variable wager determined by
the number of coins deposited in slot 18 and pay out a
prize in accordance with the amount wagered. The
central computer would know the denomination of the

10    coin and the number of coins inserted by a code number
within the identification address of each machine
terminal, for example, 1100001 would identify terminal
#1 which is set for one coin operation at a denomination
of $1.00. Should the customer insert $5.00 for a

15    single play, the address would change to 1500001. A
quarter machine with 3 coins inserted would change the
first two numbers of the address to 5300002. The first
digit of the identification number denotes the denomination
of the coin while the second digit shows the amount of

20    coins that have been inserted by the customer, for a
single play.

        In order to determine the down time of each
remote terminal 10, central computer 32 periodically
runs a monitor subroutine program during which an

25    attempt is made to communicate with each machine termi-
nal 10. Whenever one of the remote terminals 10 posts
an out-of-order light (see block 144 of Figure 5A), it
also stores an out-of-order bit in a predetermined
location in RAM memory 40. If a handshake mode cannot

30    be established or if the handshake is established but
interrogation determines that an error bit is stored in
RAM 40, central computer 32 causes this information to
be stored in a file of disc memory 78 in order that a
continuous record of the down time of each remote

35    terminal 10 may be maintained. At the same time,

central computer 32 causes a print-out of the machine
terminal which is down along with the time and date of
the interrogation.  Service personnel can then repair
the out-of-order terminal.

5            The present invention may be embodied in
other specific forms without departing from the spirit
or essential attributes thereof and, accordingly,
reference should be made to the appended claims, rather
than to the foregoing specification, as indicating the
10   scope of the invention.

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WHAT IS CLAIMED IS:

    1.   A computerized gaming system, comprising:

    (A)   a plurality of remote game machine terminals, each of said remote game machine terminals having a unique game machine terminal identification code associated therewith;

    (B)   a central processing station remotely located from said game machine terminals, said central processing station communicating with said remote game machine terminals via an information transmission channel;

    (C)   each of said remote game machine terminals including first means for transmitting a request signal to said central processing station responsive to the generation of a player activated game initiation signal, said request signal being indicative of both a player selected indicia and the identification code unique to the remote game machine terminal transmitting said request signal;

    (D)   said central processing station including second means for:

    (1)   generating a random sequence of indicia whenever said central processing station receives a request signal generated by one of said remote game machine terminals;

    (2)   generating a win code indicative of a prize amount determined by the number of matches between the player selected indicia identified by said received request signal and said random sequence of indicia in accordance with a predetermined prize structure;

    (3)   issuing a validation ticket at said central processing station if said prize amount indicated by said win code is greater than a predetermined value, said validation ticket identifying the identification code associated with said remote game machine terminal which transmitted said received request signal; and

35          (4)   transmitting a response signal to
said remote game machine terminals, said response signal
indicating said random sequence of indicia, said win code
and the identification code associated with said remote
game machine terminal which transmitted said received
40  request signal; and
          (E)   said first means also determining if said
transmitted response signal contains the identification
code unique to the respective game machine terminal and,
if said identification code is present, for:
45          (1)   displaying said random sequence of
indicia indicated by said received response signal;
          (2)   examining said win code indicated by
said received response signal to determine if said prize
amount is greater than said predetermined value; and
50          (3)   immediately paying out said prize
amount if said winnings are below said predetermined
value and providing a human perceptible indication that a
grand prize has been won if said prize amount is greater
than said predetermined value.


          2.   The computerized gaming system of claim 1,
wherein said first means includes means for encoding said
transmitted request signal in accordance with an operator
specified key and for decoding said received response
5   signal in accordance with said operator specified key.


          3.   The computerized gaming system of claim 2,
wherein said second means includes means for decoding
said received request signals in accordance with said
operator specified key and for encoding said transmitted
5   response signals in accordance with said operator specified
key.

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4. The computerized gaming system of claim 3, wherein said first and second means each include means for permitting the operator of said computerized gaming system to change said operator specified key.

5. The computerized gaming system of claim 1, wherein said first means includes:
(A) a digital display for displaying said random sequence of indicia; and
5 (B) a keyboard for enabling said player to select said player selected indicia.

6. The computerized gaming system of claim 5, wherein said digital display includes a plurality of electronic displays.

7. The computerized gaming system of claim 6, wherein said first means further includes means for annunciating those indicia of said random indicia which match said player selected indicia.

8. The computerized gaming system of claims 6 or 7, wherein said keyboard includes a plurality of push buttons and wherein said first means includes means for back lighting the said push buttons selected by said
5 player.

9. The computerized gaming system of claim 1, wherein said first means further includes means for annunciating those indicia of said random sequence of indicia which match said player selected indicia.

10. The computerized gaming system of claim 1, wherein said validation ticket also includes information regarding the date and time at which said game was played.

11.   The computerized gaming system of claims 1 or 10, wherein said validation ticket further includes information identifying said computerized gaming system.

12.   The computerized gaming system of claim 1, wherein said prize amount is determined by the number of matches between individual elements of said random sequence of indicia as well as the number of matches
5   between said player selected indicia and said sequence of indicia.

13.   The computerized gaming system of claim 1, wherein said second means also includes means for randomly generating a win code indicative of a random prize amount irrespective of the number of matches between said player
5   selected indicia and said random sequence of indicia.

14.   The computerized gaming system of claim 1, wherein said prize amount is determined in accordance with a first predetermined prize structure during certain hours of the day and in accordance with a second pre-
5   determined prize structure during other hours of the day.

15.   The computerized gaming system of claim 1, including means for enabling the operator of said computerized gaming system to change said predetermined prize structure.

16.   A method for playing a game of chance utilizing a computerized gaming system comprising a plurality of remote game machine terminals, each of said remote game machine terminals having a unique game
5   machine terminal identification code associated therewith,

and a central processing station remotely located from said machine terminals and communicating with said remote game machine terminals via an information transmission channel, said method comprising the steps of:

10    (A) transmitting a request signal from one of said remote game machine terminals to said central processing station responsive to the generation of a player activated game initiation signal, said request signal being indicative of a player selected indicia

15    and the identification code of said one of said game machine terminals;

        (B) at said central processing station;

        (1) generating a random sequence of indicia responsive to receipt of said request signal;

20        (2) generating a win code indicative of a prize amount determined by the number of matches between said player selected indicia identified by said received request signal and said random sequence of indicia in accordance with a predetermined prize structure;

25        (3) issuing a validation ticket if said prize amount indicated by said win code is greater than a predetermined value, said validation ticket identifying the identification code associated with said one of said remote game machine terminals and the prize amount

30    indicated by said win code; and

        (4) transmitting a response signal to said one of said game machine terminals, said response signal indicating said random sequence of indicia, said win code and said identification code associated with

35    said one of said remote game machine terminals; and

        (C) determining, at said one of said remote game machine terminals, if said transmitted reponse signal contains said identification code associated with said one of said game machine terminals and, if

40    said identification code is present:

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(1)  displaying, at said one of said re-
mote game machine terminals, said random sequence of
indicia indicated by said received response signal;

(2)  examining, at said one of said remote
45   game machine terminals, said win code indicated by said
received response signal to determine if said prize
amount is greater than said predetermined value; and

(3)  immediately paying out, at said one of
said remote game machine terminals, said prize amount
50   if said prize amount is below said predetermined value
and providing a human perceptible indication that a grand
prize has been won if said prize amount is greater than
said predetermined value.


17.  The method of claim 16, further including
the step of encoding said transmitted request signal in
accordance with an operator specified key and decoding
said received response signal in accordance with said
5    operator specified key.


18.  The method of claim 17, further including
the steps of decoding said received request signals in
accordance with said operator specified key and for
encoding said transmitted response signals in accordance
5    with said operator specified key.


19.  The method of claim 18, further including
the step of changing said operator specified key.


20.  The method of claim 26, further including
the step of annunciating those indicia of said random
sequence of indicia which are displayed at said one of
said remote game machine terminals and which match said
5    player selected indicia.
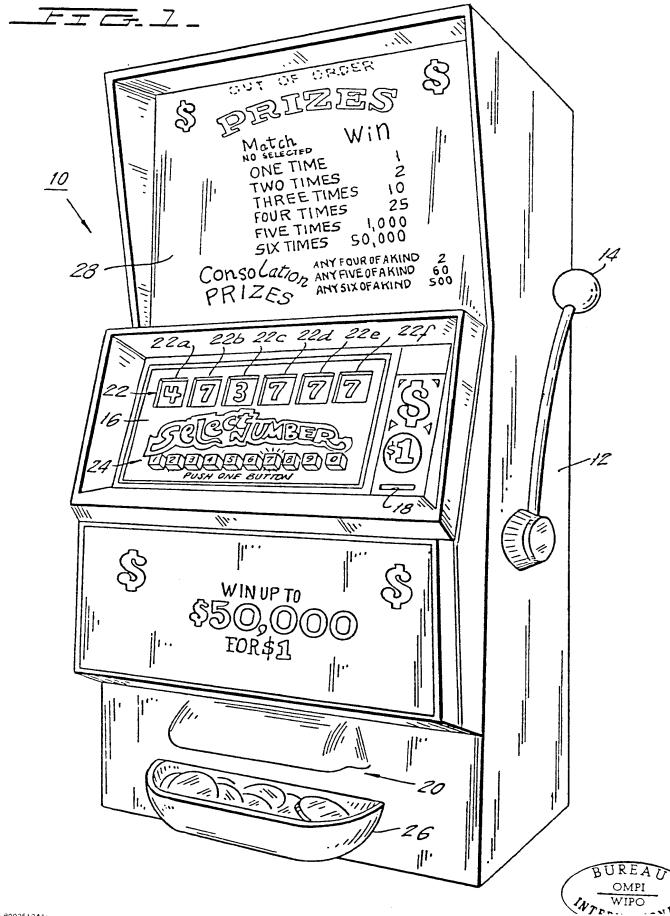
21.　The method of claim 16, wherein said
prize amount is determined by the number of matches between
individual elements of said random sequence of indicia
as well as the number of matches between said player
5　selected indicia and said sequence of indicia.

22.　The method of claim 16, further including
the step of randomly generating, at said central process-
ing station, a win code indicative of a random prize
amount irrespective of the number of matches between
5　said player selected indicia and said random sequence
of indicia.

23.　The method of claim 16, wherein said
prize amount is determined in accordance with the first
predetermined prize structure during certain hours of
the day and in accordance with the second predetermined
5　prize structure during other hours of the day.

24.　The computerized gaming system of claim 1,
wherein said first means includes:
　　　(a)　a digital display for displaying said
random sequence of indicia; and
5　　　　(b)　a dial for enabling said player to select
said player-selected indicia.

25.　The computerized gaming system of claim 1,
wherein said prize amount is normally determined in
accordance with a first predetermined prize structure and
is determined in accordance with a second predetermined
5　prize structure during random hours of the day.

26. The method of claim 16, wherein said prize amount is normally determined in accordance with a first predetermined prize structure and is determined in accordance with a second predetermined prize structure during random hours of the day.

FIG. 1.

OUT OF ORDER

PRIZES

Match WIN
NO SELECTED
ONE TIME 1
TWO TIMES 2
THREE TIMES 10
FOUR TIMES 25
FIVE TIMES 1,000
SIX TIMES 50,000

Consolation PRIZES
ANY FOUR OF A KIND 2
ANY FIVE OF A KIND 60
ANY SIX OF A KIND 500

Select NUMBER
PUSH ONE BUTTON

WIN UP TO
$50,000
FOR $1

FIG.2.

FIG. 3.

FIG. 4.

START (A)

COIN
INSERTED
METER          86
ADV.

LEVER
PULLED &
INTERRUPT      88
SIGNAL GEN

STORE
NUMBER
PICKED         90
IN CPU 36

TRANSMIT
REQUEST
SIGNAL TO      92
COMPUTER-32

BEGIN 75MS
COUNT IN
FIRST          94
DISPLAY

BEGIN 100MS
COUNT IN
SECOND         96
DISPLAY

BEGIN 95MS
COUNT IN
THIRD          98
DISPLAY

BEGIN 125MS
COUNT IN
FOURTH         100
DISPLAY

BEGIN 150MS
COUNT IN
FIFTH          102
DISPLAY

BEGIN 175MS
COUNT IN
SIXTH          104
DISPLAY

PAUSE
100 MS         106

REC.
RESPONSE       108        NO      RETURN      DISABLE
SIGNAL                            COIN        DISPLAY
                                  110         112

YES

STORE
DATA
IN RAM         131                            POST
                                    114       OUT OF ORDER
                                              LINE
                                              114

DAMP
COUNT
INCREMENT      133
ON DISPLAYS

STOP                    FIG. 5A
DISPLAY
ONE IN         135
300 MS

(B)

FIG. 5B.

_FIG.6._

START

C

RECEIVE & DECODE DATA — 116

GENERATE RANDOM NUMBER — 118

COLLECT PLAY & WIN DATA — 129

COMPUTE WIN CODE — 120

WIN OVER 250 — 122

YES → PRINT OUT PAYMENT VOUCHER — 128

NO

ENCODE DATA — 124

TRANSMIT DATA — 126

END



_FIG_7_

22a    22b

PRIZE STRUCTURE A    PRIZE STRUCTURE B

22

10

BUREAU
OMPI
WIPO

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US80/00404
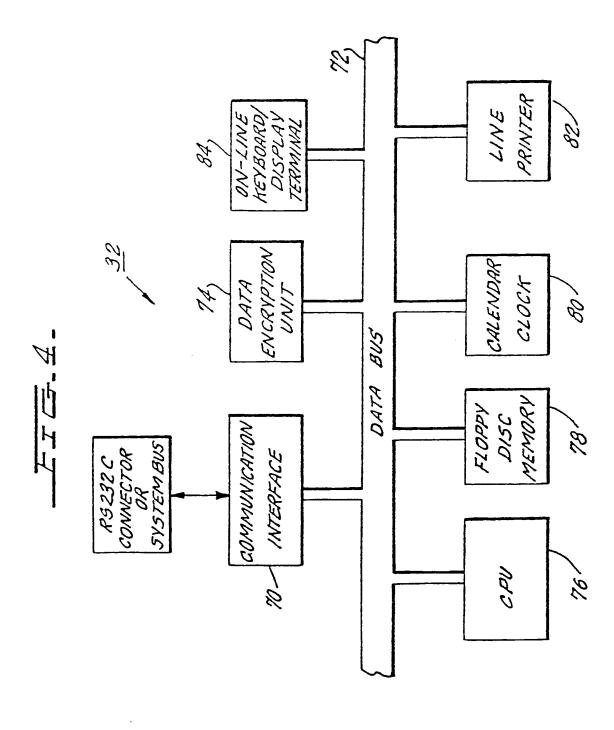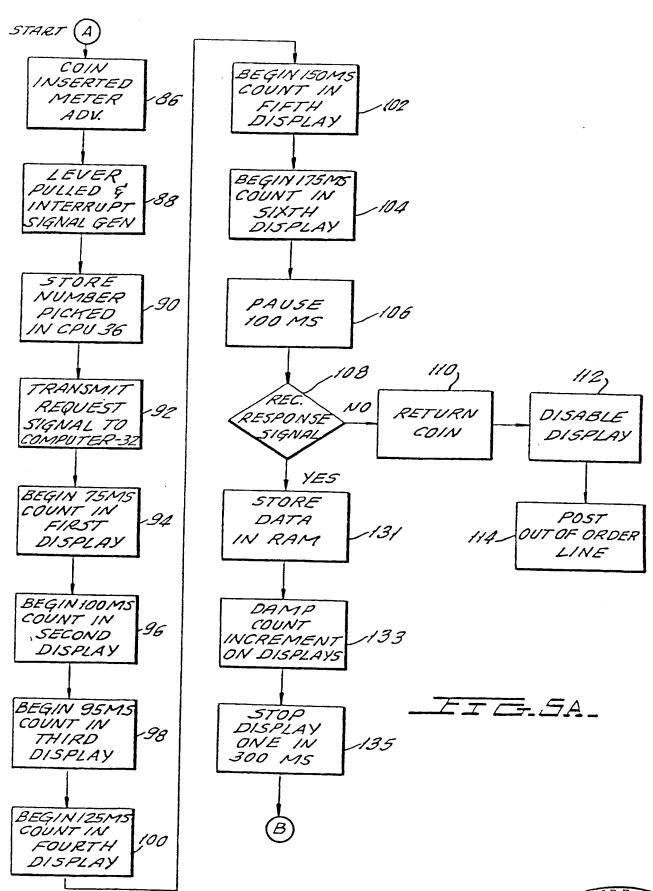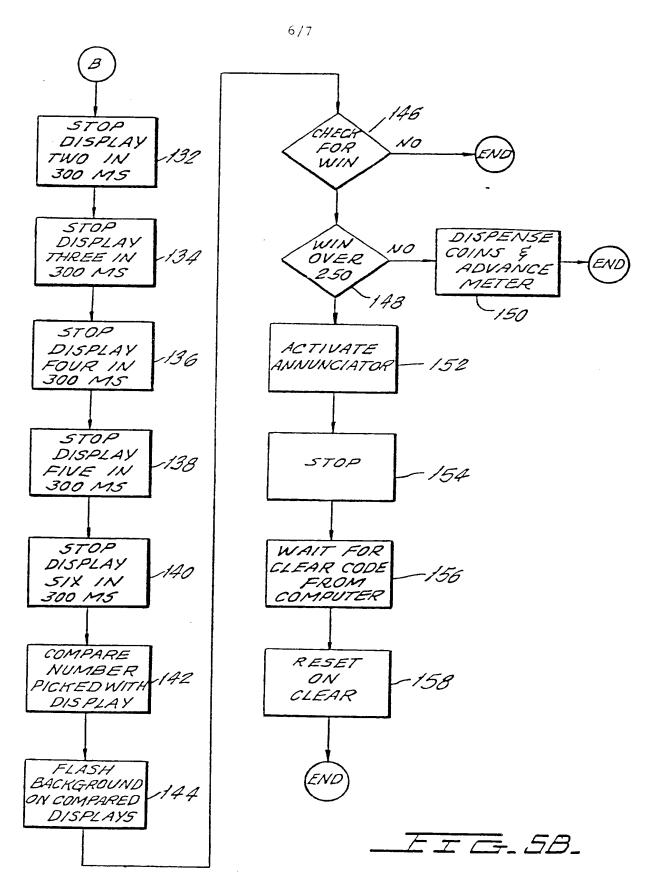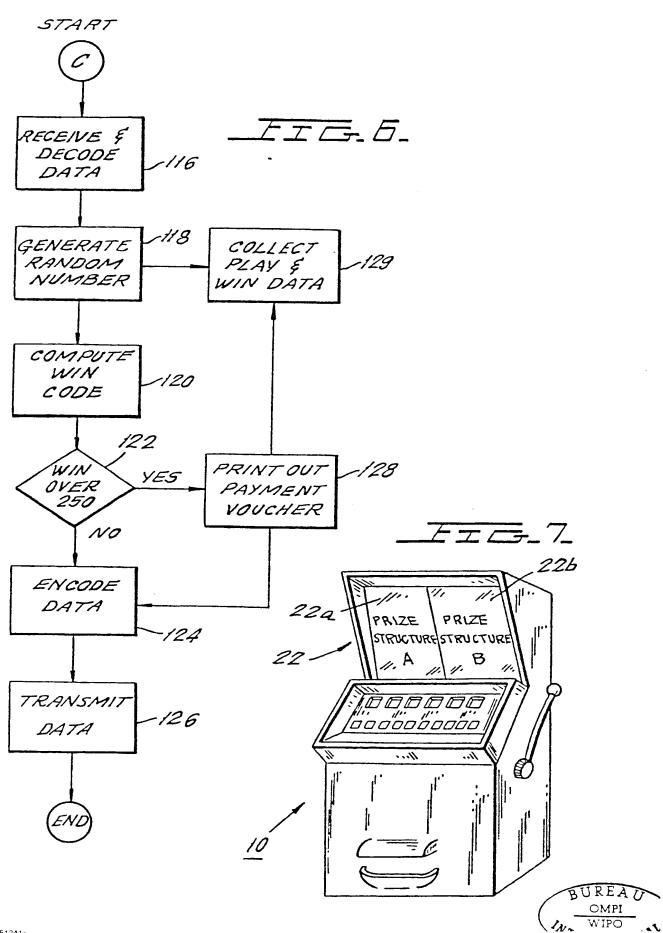
## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁸

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL³ A63F 5/04
U.S. CL. 273/138A

## II. FIELDS SEARCHED

### Minimum Documentation Searched ⁴

| Classification System | Classification Symbols |
|---|---|
| U.S. | 194/97R<br>273/1E, 138A,143R,143C<br>340/323R<br>364/412 |

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

## III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

| Category * | Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷ | Relevant to Claim No. ¹⁸ |
|---|---|---|
| X | US, A, 3,786,234, Published 15 January 1974, Trent. | 1-26 |
| X | US, A, 3,770,269, Published 06 November 1973, Elder. | 1-26 |
| X | US, A, 3,796,433, Published 12 March 1974, Fraley. | 8 |
| X | US, A, 4,072,930, Published 07 February 1978, Lucero. | 9,15,20 |
| A | US, A, 4,095,795, Published 20 June 1978, Saxton. | 1-26 |
| A | US, A, 4,099,722, Published 11 July 1978, Rodesch. | 1-26 |
| A, P | US, A, 4,157,829, Published 12 June 1979, Goldman. | 1-26 |

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search ² | Date of Mailing of this International Search Report ² |
|---|---|
| 16 September 1980 | 29 SEP 1980 |
| International Searching Authority ¹ | Signature of Authorized Officer ¹⁰ |
| ISA/US | V.Y. Hum |

Form PCT/ISA/210 (second sheet) (October 1977)

# PATENT COOPERATION TREATY

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

*in its capacity as elected Office*

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>15 February 2000 (15.02.00) | |
| **International application No.**<br>PCT/IL99/00417 | **Applicant's or agent's file reference**<br>453-A-PCT |
| **International filing date** (day/month/year)<br>29 July 1999 (29.07.99) | **Priority date** (day/month/year)<br>30 July 1998 (30.07.98) |
| **Applicant**<br><br>EISENBACH, Lea et al | |

1.  The designated Office is hereby notified of its election made:

    [X] in the demand filed with the International Preliminary Examining Authority on:

    17 January 2000 (17.01.00)

    [ ] in a notice effecting later election filed with the International Bureau on:

2.  The election  [X] was

    [ ] was not

    made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

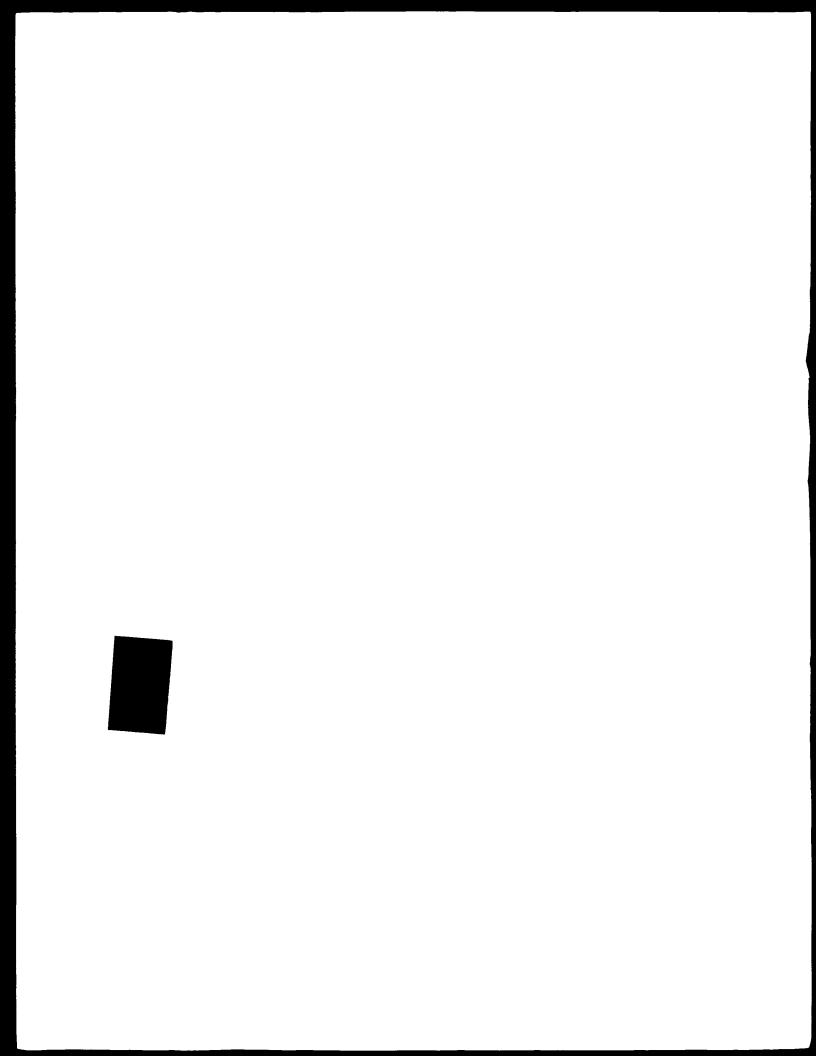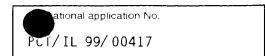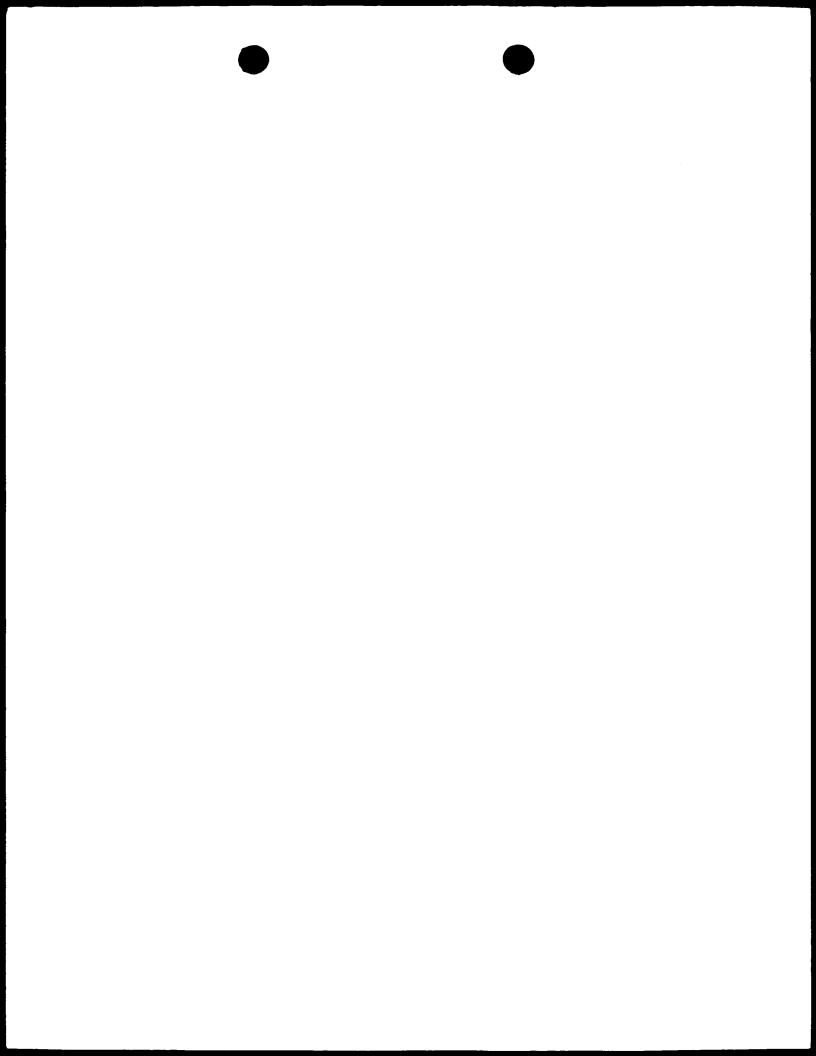| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer<br><br>Juan Cruz |
|---|---|
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

Form PCT/IB/331 (July 1992)

3107861

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL 99/00417

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claims 37-38 are directed to a method of treatment
           of the human/animal body, the search has been carried out and
           based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:       43-45
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

   see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**          [ ] The additional search fees were accompanied by the applicant's protest.

                               [ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

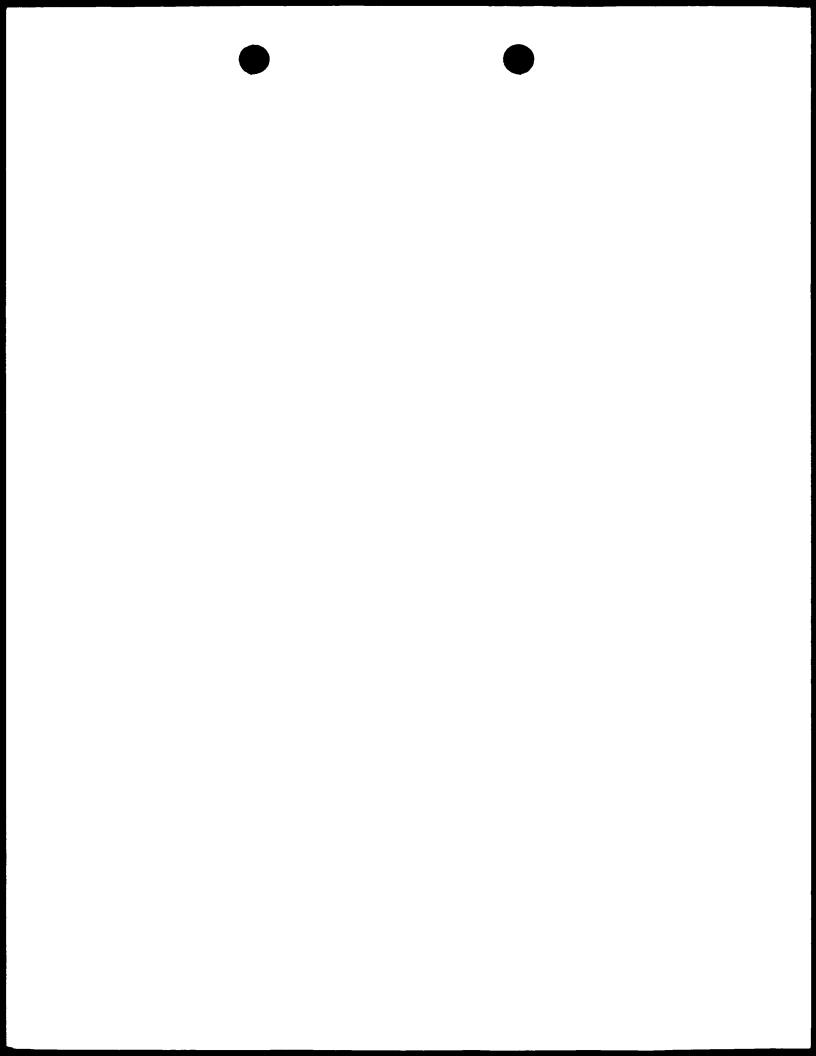**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**
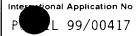
Continuation of Box I.2

Claims Nos.: 43-45

Present claims 23-25 relate to an extremely large number of possible compounds/products. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds/products prepared in the examples and closely related homologous compounds like those mentioned in the sequence listing.

Present claims 43-45 relate to an extremely large number of possible compounds/products/apparatus/methods. In fact, the claims contain so many options, variables, possible permutations and provisos that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, no search has been carried out for this parts of the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7  C12N15/12  C07K14/47  C07K14/705  C12N9/16  C12N9/64
       A61K38/17  A61K38/46  A61K38/47  C12N15/55  C12N15/57
       C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7  C07K  C12N  A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No |
|---|---|---|
| P,X | WO 98 43084 A (UNIV LELAND STANFORD JUNIOR) 1 October 1998 (1998-10-01) claims; table 4 | 1,5,6, 20-22,55 |
| X | WO 94 20127 A (CYTEL CORP) 15 September 1994 (1994-09-15) table 25 | 1,5,6, 20-22,55 |
| X | DE 195 16 673 A (PECHER GABRIELE DR) 31 October 1996 (1996-10-31) claims; examples | 1,13, 20-22, 43-45 |

-/--

[X] Further documents are listed in the continuation of box C.    [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 30 December 1999 | 12/01/2000 |

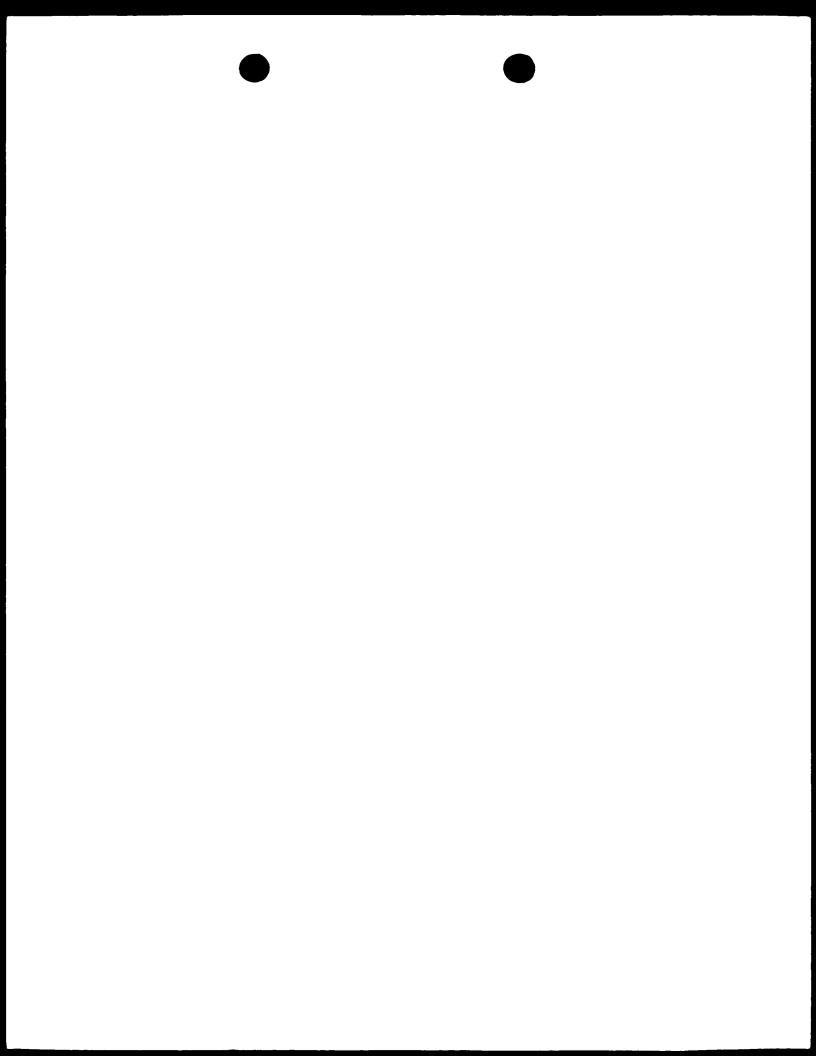| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016 | Fuhr, C |

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Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

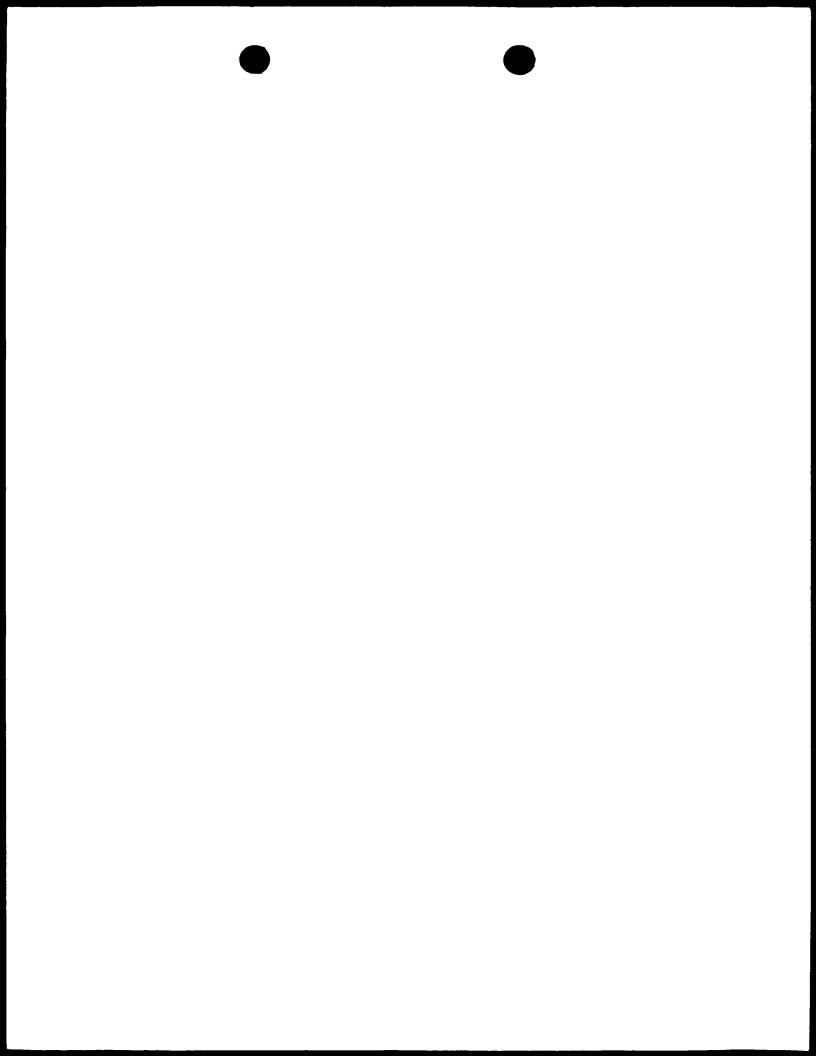| Category | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No |
|---|---|---|
| X | WO 97 08318 A (CORIXA CORP) 6 March 1997 (1997-03-06)  claims; examples --- | 1,5, 20-22, 26,30, 31,34, 51-55 |
| A | WO 95 04548 A (JENNER TECHNOLOGIES) 16 February 1995 (1995-02-16) page 7, line 17 - line 35; claims; examples --- | 1,26,32, 37,38,42 |
| P,A | DE 197 58 400 A (HANISCH FRANZ GEORG PROF DR ;MAX DELBRUECK CT FUER MOLEKULA (DE)) 1 July 1999 (1999-07-01)  claims; table 1 --- | 1,26,32, 37,38,42 |
| X | WO 97 11715 A (AUSTIN RESEARCH INST ;SANDRIN MAURO SERGIO (AU); MCKENZIE IAN FARQ) 3 April 1997 (1997-04-03)  claims; examples --- | 1,11-13, 26-38, 46,51-55 |
| X | WO 97 35021 A (US HEALTH ;ZAREMBA SAM (US); SCHLOM JEFFREY (US); TSANG KWONG YOK) 25 September 1997 (1997-09-25)  claims; examples; table 10 --- | 1,5,20, 21, 26-38, 51-55 |
| A | WO 95 19783 A (CELIS ESTEBAN ;GREY HOWARD M (US); CYTEL CORP (US); KUBO RALPH T ()) 27 July 1995 (1995-07-27) claims; examples --- | 1,26 |
| A | WEINSTEIN B: "CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS, PASSAGE" CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS,XX,XX, vol. 7, page 266-357 XP002032461 the whole document ----- | 23-25 |

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

| Patent document cited in search report | | Publication date | Patent family member(s) | | | Publication date |
|---|---|---|---|---|---|---|
| WO 9843084 | A | 01-10-1998 | US | 5858685 | A | 12-01-1999 |
| | | | AU | 6570998 | A | 20-10-1998 |
| WO 9420127 | A | 15-09-1994 | AU | 6359494 | A | 26-09-1994 |
| | | | AU | 6597998 | A | 02-07-1998 |
| | | | BR | 9406652 | A | 10-09-1996 |
| | | | CA | 2157510 | A | 15-09-1994 |
| | | | CN | 1118572 | A | 13-03-1996 |
| | | | EP | 0703783 | A | 03-04-1996 |
| | | | JP | 8507525 | T | 13-08-1996 |
| | | | NZ | 263050 | A | 24-11-1997 |
| | | | SG | 49008 | A | 18-05-1998 |
| DE 19516673 | A | 31-10-1996 | NONE | | | |
| WO 9708318 | A | 06-03-1997 | US | 5854206 | A | 29-12-1998 |
| | | | AU | 7012496 | A | 19-03-1997 |
| WO 9504548 | A | 16-02-1995 | AU | 686660 | B | 12-02-1998 |
| | | | AU | 7631294 | A | 28-02-1995 |
| | | | CA | 2168952 | A | 16-02-1995 |
| | | | EP | 0721345 | A | 17-07-1996 |
| | | | JP | 9504000 | T | 22-04-1997 |
| | | | US | 5925362 | A | 20-07-1999 |
| DE 19758400 | A | 01-07-1999 | WO | 9934824 | A | 15-07-1999 |
| WO 9711715 | A | 03-04-1997 | AU | 7079396 | A | 17-04-1997 |
| | | | AU | 707223 | B | 08-07-1999 |
| | | | AU | 7079496 | A | 17-04-1997 |
| | | | WO | 9711963 | A | 03-04-1997 |
| | | | CA | 2233447 | A | 03-04-1997 |
| | | | EP | 0859627 | A | 26-08-1998 |
| WO 9735021 | A | 25-09-1997 | AU | 2535997 | A | 10-10-1997 |
| | | | EP | 0888456 | A | 07-01-1999 |
| WO 9519783 | A | 27-07-1995 | US | 5662907 | A | 02-09-1997 |
| | | | AU | 1834095 | A | 08-08-1995 |
| | | | AU | 4111999 | A | 23-09-1999 |
| | | | CA | 2181920 | A | 27-07-1995 |
| | | | EP | 0749315 | A | 27-12-1996 |
| | | | SG | 49743 | A | 15-06-1998 |

# PATENT COOPERATION TREATY

## PCT

**9/744804**

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference<br><br>453-A-PCT | **FOR FURTHER ACTION** | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
|---|---|---|---|
| International application No.<br><br>PCT/IL 99/00417 | International filing date *(day/month/year)*<br><br>29/07/1999 | | (Earliest) Priority Date *(day/month/year)*<br><br>30/07/1998 |

| Applicant<br><br>YEDA RESEARCH AND DEVELOPMENT COMPANY....et al. |
|---|

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of _____5_____ sheets.

[X]   It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ]   the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X]   contained in the international application in written form.

   [X]   filed together with the international application in computer readable form.

   [ ]   furnished subsequently to this Authority in written form.

   [ ]   furnished subsequently to this Authority in computer readble form.

   [ ]   the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ]   the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.   [X]   **Certain claims were found unsearchable** (See Box I).

3.   [ ]   **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X]   the text is approved as submitted by the applicant.

   [ ]   the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X]   the text is approved as submitted by the applicant.

   [ ]   the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ]   as suggested by the applicant.                     [X]   None of the figures.

   [ ]   because the applicant failed to suggest a figure.

   [ ]   because this figure better characterizes the invention.

Form PCT/ISA/210 (first sheet) (July 1998)